STUDIES OF THE SOIL-PLANT RELATIONSHIPS OF COPPER, MOLYBDENUM AND SULPHUR IN HILL PASTURES

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DECLARATION

I declare that the composition of this thesis and the investigations presented herein are my own work.

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ABSTRACT

This project investigated the soil-plant relationships of copper (Cu), molybdenum (Mo) and sulphur (S) in hill pastures following observations of increased incidence of Mo and S-induced negative Cu balances in sheep grazing some hill sites after improvement. Although it is a relatively simple procedure to dose the sheep with Cu to prevent the development of negative Cu balances, associated with the risk of swayback and reduced lamb growth rates, it would be advantageous if sufficient were known about the soil-plant relationships of Cu, Mo and S to modify hill improvement practices and choice of sites, so that the problem does not arise.

Twenty-four Scottish hill soils were collected and analysed for various physical and chemical characteristics and on the basis of these results nine soils were chosen for further study. The nine soils, in particular soil 2 (a brown earth from HFRO Glensaugh) and soil 8 (a peaty podzol from HFRO Sourhope) were used in a series of glasshouse pot experiments in which the various processes undertaken during hill improvement were investigated: drainage, liming, fertilization with N, P and Cu and the replacement of native grasses with perennial ryegrass (PRG) and white clover (WC).

Soils 2 and 8 were incubated under waterlogged conditions. This had little effect on extractable soil Cu or Mo concentration in soil 8 but slightly decreased the former (Cu) and greatly increased the latter (Mo) in soil 2; for both soils extractable SO_4^{2-}-S concentrations decreased. When PRG was grown in the two soils which had previously been maintained in a dry, moist or waterlogged state, the effect of pre-treatment influenced the soil and plant Cu, Mo and S concentrations as did the water content at which the plants were grown. However, the effects varied with the combination of soil, pre-treatment and soil water content.

Application of lime to a soil generally had little effect on extractable soil Cu concentration at low pH but slightly increased them at higher pHs, whereas plant Cu concentration decreased as pH increased. Both plant and extractable soil Mo and S contents were increased. The method of lime application (surface or applied throughout the soil) did not alter the effects of lime addition.

Application of Cu did not alter plant or extractable soil Mo or S concentrations, but increased those of Cu. Nitrogen application as either NH_4^+ or NO_3^- had little effect on plant Cu concentration but slightly decreased those of Mo and S; the effects were similar for both forms of N. Phosphorus application had only a small influence on plant or extractable soil Cu and Mo concentrations but decreased plant S contents while not affecting extractable soil SO_4^{2-}-S contents.

Generally, WC contained more Cu and Mo in herbage than did PRG but this was reversed for herbage S concentration.

Soil type had a very strong influence on the effects produced by the various processes investigated.

The percentage (A_{Cu}) and absolute (T_{Cu}) availabilities of Cu to sheep were calculated to assess how the different processes studied would affect the Cu balances in the diet of animals - 80% of the values collected during the course of this study would probably have given rise to negative Cu balances. It was concluded that until further work is carried out on soil physico-chemical and plant morphological aspects of the soil-plant relationships of Cu, Mo and S in hill pastures, and in particular until field trials are undertaken, it will not be possible to predict accurately which hill sites will, when improved, cause negative Cu balances in sheep.

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Introduction and Literature Review

1. BACKGROUND TO AREA OF STUDY

Swayback is a nervous disorder of newborn and young lambs, caused by an induced copper deficiency, which is characterised by unco-ordinated movement of the hind quarters (Underwood, 1977). The incidence of the disease in sheep flocks has been observed to increase when hill pastures are improved; and recently work by Whitelaw *et al.* (1977, 1979) at HFRO Sourhope, has demonstrated that hill pasture improvement may also lead to reduced lamb live weights, again because of an induced copper deficiency. The financial loss attributable to these conditions has been estimated to be £1.3 million per year (MAFF, 1982). The research reported in this thesis was undertaken to try to understand why the occurrence of induced copper deficiency in animals may increase when hill pastures are improved.

Both of the disorders mentioned above occur although there is apparently sufficient copper in the diet, as the sheep is unable to utilize the copper present because of an antagonistic interaction with molybdenum and sulphur, that is there is an *induced* copper deficiency (Dick, 1956; Suttle, 1974, 1975). It is believed that the rumen microflora mediate a reaction between the sulphur and molybdenum forming thiomolybdates (Dick *et al.*, 1975), probably tetrathiomolybdates (Mills *et al.*, 1978), which complex with the dietary copper decreasing its absorption and utilization.

It is the relative concentrations of copper, molybdenum and sulphur in the sheep's diet that are important and not their absolute values. Suttle (1983b) used a copper repletion technique with Scottish Blackface sheep to derive an equation relating the absorbability of copper by the animal to the levels of molybdenum and sulphur in grazed summer herbage.

$$\text{Cu absorption (\%)} = 5.71 - 1.279S - 2.785 \ln Mo + 0.227 Mo \times S \quad \ldots \textit{(1)}$$
$$(r = 0.90,\ 9\ d.f.)$$

where S and Mo are the herbage concentrations of sulphur and molybdenum in g kg^{-1} DM and mg kg^{-1} DM, respectively. From Figure 1.1 it can be seen that as both sulphur and molybdenum concentrations in the herbage increase, the absorption of copper by the sheep decreases.

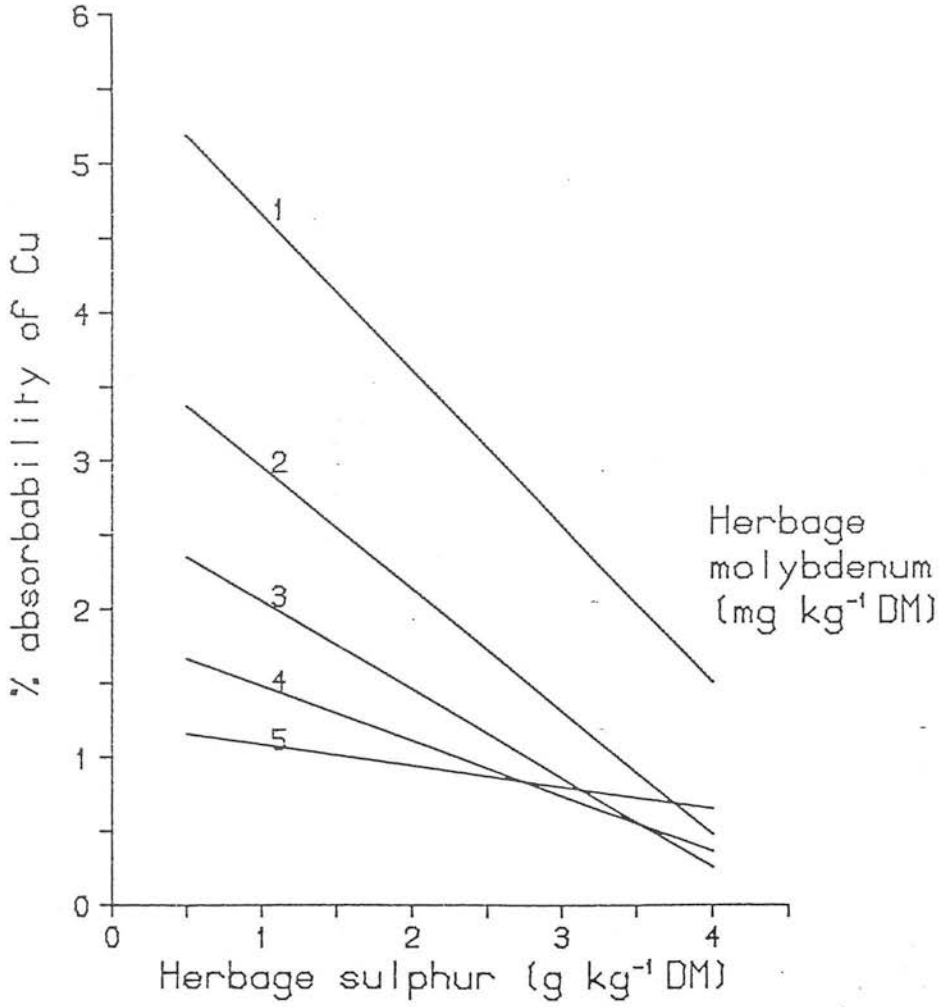

Figure 1.1: The effect of dietary molybdenum (mg kg^{-1} DM) and sulphur concentrations (g kg^{-1} DM) on the percentage of dietary copper absorbable by sheep, as predicted by equation *(1)* (Suttle, 1983b).

Equation *(1)* gives a better prediction of the absorbability of copper from grazed summer pasture than the equation previously derived by Suttle (1979) using semi-purified diets, but it should not be extrapolated to herbage concentrations of molybdenum greater than 6 mg kg^{-1} DM or of sulphur greater than 4 g kg^{-1} DM.

An indication of the effect that hill pasture improvement has on the absorbability of copper by sheep can be seen from Table 1.1, from Evans (1984). If these values for absorbable herbage copper are compared with published values of the minimum copper requirement for sheep (ARC, 1980) and with absorbable herbage copper concentrations that have been found to give rise to induced copper deficiencies in grazing lambs (Whitelaw *et al.*, 1979, 1983), it is apparent that whereas the improved pasture provides only about 25% of the dietary copper requirements for grazing lambs, the unimproved pasture provides sufficient.

Table 1.1: Concentrations of copper (Cu), molybdenum (Mo) and sulphur (S) in herbage; and calculated percentage and concentration of herbage copper absorbable by sheep, for unimproved and improved hill pastures (from Evans, 1984).

Pasture type	Concentrations of nutrients (mg kg^{-1} DM)			% Absorbability of copper	Concentration of absorbable herbage Cu (mg kg^{-1})
	Cu	Mo	S		
	(a)			*(b)*	*(a x b)*
Unimproved	6.0	0.8	0.18	4.5	0.27
Improved	5.2	6.2	0.30	1.0	0.053

The literature was surveyed for information about copper, molybdenum and sulphur in soils and plants, and the effect that various factors have on the soil-plant relationships of the three elements (for example, pH, soil water status and organic matter). The literature

relevant to the alteration of the relative concentrations of copper, molybdenum and sulphur following the improvement of hill pastures has been reviewed here, with each of the three elements being considered separately. A summary table of the effect that the various factors are reported to have on the three elements is presented in Table 1.6. Two recent publications which have briefly covered some areas of this literature review are 'Copper in ruminant animal nutrition' (MAFF, 1982), and 'Trace element deficiency in ruminants' (Scottish Agricultural Colleges and Scottish Agricultural Research Institutes, 1982).

2. COPPER, MOLYBDENUM AND SULPHUR IN PLANTS AND SOILS

2.1 Soil copper

(a) Soil content

The most important factor in determining the trace element content of the relatively young unweathered soils of the U.K. is the parent material; pedological factors including gleying, podzolization, surface organic matter accumulation, pH and redox potential will also affect the content, but to a lesser extent (Swaine and Mitchell, 1960). Soils developed from coarse grained materials (sands or sandstones) or from acid igneous rocks have lower total trace element concentrations than soils developed from fine grained sedimentary rocks (clays or shales) or from basic igneous rocks (Swaine and Mitchell, 1960; Mitchell, 1964).

The relationship between the total Cu content of a soil and its parent material has been examined by Mitchell (1971) for Scottish surface soils: <10 mg kg^{-1} from trachyte, granite or sandstone; 10 mg kg^{-1} from andesite or shale; 20 mg kg^{-1} from serpentine; 25 mg kg^{-1} from granitic gneiss; 40 mg kg^{-1} from quartzite or olivine gabbro; and 100 mg kg^{-1} from quartz mica schist. The normal range of total soil Cu for

Scotland is 1-50 mg kg^{-1}, mean 20 mg kg^{-1} and there is a slight tendency for the concentration to increase with depth of the soil profile (Mitchell *et al.*, 1957a; Swaine and Mitchell, 1960). Despite the wide variability of soil Cu which arises because of the very diverse nature of glaciated Scottish soils, Cu deficient soils, i.e. those with <2 mg kg^{-1} total Cu (Purves and Ragg, 1962) are comparatively common, as demonstrated by Reith (1968) for north-east Scotland and Purves and Ragg (1962) for south-east Scotland.

The usual range of EDTA-extractable Cu in Scottish soils is 0.3-10 mg kg^{-1} (Mitchell, 1964). Mitchell (1963) considers that soils with <0.8 mg kg^{-1} EDTA-extractable Cu are liable to give rise to Cu deficiency in cereals; for pastures, soils containing <1.2 mg kg^{-1} are considered low in copper (Mackenzie, 1974).

(b) Form in soil and adsorption on soil surface

Specific/non-specific adsorption - If ions do not have a special affinity for a surface they will be adsorbed in proportion to their concentration in the soil solution - *non-specific adsorption*. If ions have a special affinity for a surface, *specific adsorption* will occur where the ions approach very close to the surface and are adsorbed to a greater extent than would be expected from their concentration in the soil solution, either through the formation of an *ion-pair* or by *ligand exchange*. Ligand exchange is the displacement of an OH or $(OH_2)^+$ group from the coordination shell of a surface metal atom. An ion-pair is the association of a positively charged ion with a negatively charged ion (White, 1979).

Surfaces of variable charge - The oxide surfaces and clay mineral edges in soil show pH-dependent charge due to reversible adsorption

of potential determining H^+ ions, the pH at which the net surface charge is nil is the *point of zero charge* (PZC). The PZC will depend on the ability of the surface to act as an acid - lose a proton, for example, silica has PZC <pH 2, whereas for ferric oxide PZC ≈pH 9. At pHs below the PZC the surface will carry a net positive charge but the magnitude of this will also depend on the ionic strength of the soil solution, the more concentrated it is the higher the charge will be (Barrow, 1975; White, 1979). Interestingly, because of the adsorption of organic matter (OM), soil hydr oxides will generally bear a net negative charge, even below their PZC - at these pHs pure oxides would bear a net positive charge (James and Barrow, 1981).

Organic matter also shows pH-dependent charge because of dissociation of carboxylic and phenolic groups, but above pH 3 the net charge is always negative (White, 1979).

McLaren and Crawford (1973a) fractionated total soil Cu to obtain:

	% of total
• Soil solution and exchangeable Cu • Cu weakly bonded to specific sites	1-2
• Organically bound Cu	30
• Cu occluded by oxide material	15
• and reduced Cu in clay lattice structures	50

McBride (1981) has reviewed how Cu is held in a soil so only a brief account will be given here, concentrating mainly on the first two fractions above - that is, the plant available Cu.

Many of the earlier studies on Cu adsorption were performed at unrealistically high solution Cu concentrations (e.g. McLaren and Crawford, 1973a, 1973b, 1974). So the results obtained may not be directly applicable to the soil where only a small proportion of the total

possible adsorption sites are utilized, for example <2% (McLaren *et al.*, 1983a), and specific adsorption rather than cation exchange will be predominant adsorption mechanism for Cu (Williams, 1981).

Recent work at realistically low solution Cu concentrations has shown that adsorption follows a linear isotherm, for soil (Jarvis, 1981b; McLaren *et al.*, 1983a) and for individual soil components (McLaren *et al.*, 1981). At higher, non-realistic, solution Cu concentrations adsorption has been observed to be non-linear and follow the Freundlich (e.g. Jarvis, 1981b) or Langmuir isotherm (e.g. McLaren and Crawford, 1973b). Only a small amount of desorption of the adsorbed Cu occurs which implies that adsorption is irreversible or only very slowly reversible, with a high activation energy necessary for desorption (McLaren *et al.*, 1983b). In studies with geothite at pH <7.5, Padmanabham (1983) has observed "hysteresis" of adsorption and desorption which he has attributed to the existence of two different adsorption sites, one with a low bonding energy where Cu^{2+} is associated with one hydroxyl group and the other with a higher bonding energy where Cu^{2+} is associated with two hydroxyl groups and forms a bridging ligand. Desorption is important in controlling the plant availability of soil Cu - both added and native.

The relative importance of the various soil surfaces in controlling the specific adsorption of Cu is organic matter $\geq$ soil oxides > clay minerals (Williams, 1981). The first two groups are usually the most important (McLaren *et al.*, 1983a), particularly manganese oxides (McLaren and Crawford, 1973b), but this will vary with soil pH which will not only determine the net charge of these surfaces but will also determine the hydrolysis state of the copper (Jarvis, 1981b).

The concentration of Cu in the soil solution is normally 0.003-0.06 mg l^{-1} (Williams, 1981), but only a small percentage of this will be present as Cu^{2+} ions and available for adsorption. The major part will be present as soluble organic complexes (McBride and Blasiak, 1979). For a calcareous soil, Hodgson *et al.* (1966) demonstrated that >98% of the solution Cu was organically bound, whilst for a neutral soil, Cavallaro and McBride (1978) found 80% of the solution Cu was complexed.

2.2 Plant copper

(a) Uptake

Mechanism: The supply of Cu to plants appears to be more dependent on root interception than on either mass flow or diffusion (Oliver and Barber, 1966; Jarvis and Whitehead, 1981). Cu is most probably taken up as the uncomplexed Cu^{2+} ion (Dragun *et al.*, 1976; Harrison *et al.*, 1984), although organic complexes influence the amount absorbed with both size (Mercer and Richmond, 1970; Petruzzelli and Guidi, 1976) and charge (Coombes *et al.*, 1977) being important. The specific adsorption of Cu^{2+} onto cell walls is an important preliminary step to absorption (Hiatt *et al.*, 1963) and it is possibly at this stage that Cu-OM complexes are involved (Graham, 1981).

Rate: Jarvis and co-workers (Jarvis, 1978, 1980, 1981b, 1981c; Jarvis and Whitehead, 1981, 1983; Jarvis and Robson, 1982) have studied the rate of Cu absorption by plants and have made some interesting observations. In flowing solution cultures, white clover (WC) and perennial ryegrass (PRG) (Jarvis, 1980), and red clover, PRG and wheat (Jarvis and Robson, 1982) were found to have similar rates of Cu absorption per unit root (mg Cu kg^{-1} dry root day^{-1}) and Cu transport from root to shoot. Therefore, any species differences in shoot Cu

concentration will be due to different growth patterns; clovers generally have relatively large root and smaller shoot systems than grasses, with correspondingly higher shoot Cu content (Jarvis, 1980). However, in a study with 21 soils, Jarvis and Whitehead (1981, 1983) showed that the rate of Cu absorption was greater for WC (range 51-125 mean 91 mg Cu kg^{-1} dry root day^{-1}) than for PRG (range 25-59 mean 36 mg Cu kg^{-1} dry root day^{-1}) which could account for higher clover shoot Cu concentrations compared with grass.

(b) Distribution in the plant

A further interesting point to emerge from the work of Jarvis and co-workers is the retention of Cu within plant roots in both solution culture (Jarvis, 1980, 1981c; Jarvis and Robson, 1982; also Tills and Alloway, 1981a) and in soil (Jarvis, 1978; Jarvis and Whitehead, 1981, 1983). Retention ranges from 14 to 96% of total Cu taken up, depending on environmental conditions and species, WC retains less than PRG (Jarvis and Whitehead, 1983); and the Cu appears to occur in the cell walls in association with organic ligands (Jarvis, 1978, 1980).

An alternative method of retention has been proposed by Mazzolini *et al*. (1983), who observed discrete, randomly distributed zones of Cu accumulation in Wimmera ryegrass roots grown in solution culture and suggested that coprecipitation of Cu with iron(III) phosphate or iron (III) oxyhydroxide had occurred. Mazzolini *et al*. also suggest that the apparent retention of Cu by plant roots is an artefact of the relatively high concentrations of Fe, P and Cu found in solution culture. However, as mentioned above, Jarvis and co-workers have also observed retention in soil culture although at slightly lower percentages, e.g. 47% for PRG in soil compared with 71% in solution culture (Jarvis, 1980; Jarvis and Whitehead, 1983).

The Cu that is present in the plant shoots is not equally distributed between different plant parts and some will contain more than others; the relative levels will depend on species. Fleming and Murphy (1968) found the Cu concentration of heads > leaves > stem for Italian ryegrass, whilst for *Poa trivialis* the order was leaves > stem > head.

(c) Relationship between plant nitrogen and copper contents

A strong correlation exists between plant N and Cu contents (Gladstones *et al.*, 1975) with the Cu existing largely as chelates with amino acids (Jarvis, 1978; Loneragan *et al.*, 1980). This association of N and Cu leads to Cu having 'variable mobility' in the phloem (Loneragan *et al.*, 1980), that is Cu is released when proteins undergo hydrolysis at senescence (Hill *et al.*, 1979a, 1979b), but otherwise remains immobile. It is therefore possible for the oldest leaves of a plant to be Cu sufficient while the youngest leaves are Cu deficient (Loneragan *et al.*, 1980).

2.3 Soil molybdenum

(a) Soil content

Soil parent material is the major factor determining the amount of total Mo as it is for Cu. Mitchell (1971) found the following concentration for Scottish surface soils: <1 mg kg^{-1} from andesite, granite, granitic gneiss, slate or sandstone, 1 mg kg^{-1} from serpentine or quartzite, 2 mg kg^{-1} from olivine gabbro, 3 mg kg^{-1} from trachyte and 5 mg kg^{-1} from quartz-mica schist. The normal range of total soil Mo for Scotland is 0.2 - 5.0 mg kg^{-1}, mean 2.0 mg kg^{-1} (Swaine, 1955) which is evenly distributed within the soil profile (Swaine and Mitchell, 1960). The levels of extractable Mo (mean 0.2 mg kg^{-1}) will vary with the depth

of soil profile depending on the drainage status of the soil - decreasing with depth in freely drained and podzolic soils and increasing to the gleyed layer in poorly drained soils (Swaine and Mitchell, 1960).

(b) Form in soil and adsorption on soil surfaces

Mo may occur in the soil in one of five forms (Gupta and Lipsett, 1981):

- water soluble molybdate in soil solution,
- organically complexed Mo,
- molybdate adsorbed on positively charged surfaces,
- discrete, secondary compounds, either crystalline or amorphous,
- and primary crystalline materials.

The Mo most readily available for plant uptake is that in the first two groups (Gupta and Lipsett, 1981). The mechanism of Mo adsorption in soils will be discussed together with sulphate adsorption as both are present as anions and are adsorbed in a similar manner.

2.4 Plant molybdenum

Root interception and mass flow are the most important mechanisms controlling the movement of the molybdate ion (MoO_4^{2-}) to plant roots for absorption (Gupta and Lipsett, 1981).

The concentration of Mo in the plant will vary with plant part; for grasses, leaves > head > stem whilst for clovers stem ≧ head and leaves (Fleming, 1963).

2.5 Soil sulphur

(a) Soil content

The total S level in world soils ranges from 30 to 1000 mg kg^{-1}, mean 700 mg kg^{-1} (Lindsay, 1979), and generally soils of a calcareous origin contain the higher levels (Williams *et al.*, 1960). In humid/temperate soils over 90% of total soil S may be present in OM and be available to plants only by mineralization (Murphy, 1980). Scottish soils contain phosphate-extractable sulphate concentrations within the range 2-60 mg kg^{-1}, most of these soils contain <12 mg kg^{-1}, a level which is inadequate for optimum plant growth without additional input of S from other sources such as fertilizers and precipitation (McLaren, 1975, south-east Scotland; Scott and Munro, 1979, north Scotland). Rainfall probably contributes 10-15 kg S ha^{-1} yr^{-1} to Scottish soils (Scott, 1976).

(b) Form in soil

Soil S may be divided into two fractions - organic and inorganic - which are in dynamic equilibrium with each other. These fractions may be further subdivided (Metson, 1979):

1. *Organic (90% of total S)*

   HI-reducible S (50% of organic S - bonded indirectly to carbon, largely ester sulphates);

   carbon-bonded S (50% of organic S - includes the fraction reducible by Raney nickel which exists mainly in amino acids and contributes 56% of C-bonded S ≃ 30% total soil S (Freney *et al.*, 1970)).

2. *Inorganic (10% of total S)*

   primary mineral S;

   elemental S (a brief intermediary in microbial transformations);

   sulphide-S (particularly common in reducing conditions);

   occluded sulphate-S ($CaSO_4$ coprecipitated with $CaCO_3$, can be up to 95% of total soil S in calcareous soils (Williams and Steinbergs, 1964));

insoluble sulphate-S (e.g. precipitated as Ba sulphate and complex basic Fe or Al sulphates);

water soluble sulphate-S (very little in well drained sites);

sulphate-S adsorbed onto soil colloids.

The adsorbed sulphate-S is the form that is chiefly available to plants which concentrate it so that S is present in macro-nutrient quantities although present in soils only at trace levels.

(c) Adsorption on soil surfaces

Since soil is a heterogeneous medium the adsorption of molybdate and sulphate anions, like that of Cu cations, is complex and not easily modelled and understood. Factors contributing to this complexity are the involvement of both specific and non-specific adsorption on soil oxides, clay minerals and OM, interactions with other ions (see page 36), and the existence of a slow adsorption reaction after the initial rapid one (Barrow, 1973). The order of specific adsorption for anions is $PO_4^{3-} > MoO_4^{2-} > SO_4^{2-} > Cl^- > NO_3^-$ (Parfitt, 1978).

The most important surfaces for adsorption are those of Fe, Al and Mn oxides in whatever form they are present, as free oxides or hydroxides, as surface coatings or within a crystal lattice (Mo - Parfitt, 1978; S - Chao *et al.*, 1962). Scott (1976) observed Fe oxides to be the major adsorbent for sulphate, possibly due to the formation of binuclear bridging complexes (Parfitt, 1978). Jones and Smith (1972) also found Fe oxides to be the major adsorbent for molybdate but McKenzie (1983) could find no evidence for this, the amount of adsorption depended solely on the available surface area.

The mechanism of specific adsorption is most probably ligand exchange of the anion with a hydroxyl group (Chao *et al.*, 1962; Hingston *et al.*, 1972), although for sulphate the formation of complex

insoluble Fe or Al hydroxysulphates (alunite, basaluminite) has been proposed (Adams and Rawajfih, 1977; Wolt, 1981). As the pH of the system increases and the amount of positive charge on the oxide surfaces decreases so adsorption of the anions will decrease (Mo - Karimian and Cox, 1978; S - Harward and Reisenauer, 1966).

Maximum adsorption of molybdate and sulphate occurs close to the pK_a of the associated acid - pK_2 H_2SO_4 = 1.9, pK_1 H_2MoO_4 = 3.6, pK_2 H_2MoO_4 = 4.2 (Hingston *et al.*, 1968). Two theories have been proposed to explain this, either that at this pH the energy required to remove one proton from the acid is at a minimum (Hingston *et al.*, 1972) or that at this pH the balance between the positive potential charge on the adsorbing surface and the negative charge of the anions is optimal (Bowden *et al.*, 1977). As the pK_2 of H_2SO_4 is very low, SO_4^{2-} will be the predominant species in the soil solution, whereas for molybdate with higher pK_a values, this will not be so and the ion will be involved in several species - $HMoO_4^-$, MoO_4^{2-}, paramolybdate $Mo_7O_{24}^{6-}$ (Reyes and Jurinak, 1967) and even $(Mo_2O_5(OH))^+$ at low pH (McKenzie, 1983); so adsorption can occur onto negatively as well as positively charged surfaces (Barrow, 1975).

The adsorption of molybdate and sulphate is not adequately described by the Langmuir or Freundlich isotherms, although both have been used (e.g. Mo, Langmuir - Barrow, 1970; Mo, Freundlich - Karimian and Cox, 1978; S, Langmuir - Aylmore *et al.*, 1967; S, Freundlich - Chao *et al.*, 1962). A better model to describe anion adsorption is that of Bowden *et al.* (1977, 1980) which takes into account the alteration of surface charge of the adsorbent with adsorption and allows the adsorbed ion to lie within the Stern layer.

Hysteresis-like effects have been observed between the adsorption and desorption of molybdate and sulphate and the existence of energetically different sites for adsorption, with slow conversion of the anion to an unavailable form has been proposed (Mo - Barrow and Shaw, 1974, 1975; S - Aylmore *et al.*, 1967).

2.6 Plant sulphur

The main source of plant S is that taken up from the soil as the sulphate anion in an active process, the mechanism of which is not known (Mengel and Kirkby, 1982). The sulphate may be derived from mineralization, from inorganic fertilizers, and in part from atmospheric deposition. Direct foliar uptake of S from the atmosphere also occurs and the total quantity of atmospheric S absorbed directly and indirectly represents a significant fraction of the S inputs to plants in the U.K. (Cowling and Jones, 1970; Jones *et al.*, 1972; Kerr, 1981). This proportion has increased as the S content of fertilizers has declined, because of the replacement of ammonium sulphate and single superphosphate by other forms.

S has restricted mobility in plants (Bouma, 1975). Translocation of S within the plant is mainly upwards (acropetal); very little is moved down (Mengel and Kirkby, 1982).

3. HILL LAND IMPROVEMENT

One-third of Britain's agricultural area lies in the hills and uplands, yet these regions contribute <10% of total agricultural production (Jones, 1978). Five factors are responsible for this limited output - climate, site, soil, vegetation and management (reviewed in HFRO, 1979). The last three, but not the first two of these factors, can be modified to

achieve signficiant increases in output (HFRO, 1979) as in the two-pasture system (Eadie, 1971, 1978). The 'two-pasture' system is a grazing management plan in which small areas of improved hill pasture are utilized strategically at nutritionally critical times during the sheep's annual cycle of production.

(a) Hill soils

U.K. hill soils can be divided into four main groups, each associated with a characteristic indigenous vegetation (see Table 1.2).

Table 1.2: The main hill soil and vegetation types in the U.K.

Soil type	Soil characteristic	pH	Vegetation
Brown earth	Mineral soils of good structure	5.2-5.5	Acid grassland (species rich)
		4.6-5.2	Acid grassland (species poor)
Gleys, peaty gleys	Impeded drainage	4.0-5.2	Grass heath
Podzols, peaty podzols	Highly leached soil of poor structure	3.8-4.5	Shrub heath
Blanket peat		<4.0	Bog

(after Newbould, 1976; Newbould and Floate, 1979)

More detailed descriptions of these soil types can be found in Pyatt (1970), Floate (1977) or Ball (1978).

Hill soils are characterized by having a poor physical structure, low pH and high OM content which lead to low levels of available nutrients because of fixation of nutrients on Fe and Al oxides and slow rates of mineralization of OM (HFRO, 1979; Newbould and Floate, 1979). The low nutrient status of the soil together with the frequently poor drainage

and short growing season of hill sites gives rise to indigenous vegetation that is of poor quality - low DM production and low digestibility (HFRO, 1979).

(b) Techniques of improvement

The objective of hill land improvement is to raise the quality of the hill pasture, usually by amelioration of the soil and introduction of more productive plant species. The technique of improvement used may range from the provision of a fence only to full cultivation with fertilizer applications and species replacement - *reseeding*; the method used in a given situation will depend on soil, vegetation, site and financial considerations.

The basic procedures of reseeding, prior to sowing the new species, are: enclosure of the area; removal of the existing vegetation by grazing, ploughing, burning or herbicide application; the addition of P (30-80 kg ha^{-1}); the addition of lime (2.5 - 7.5 t ha^{-1}) to reduce Al toxicity, and to raise the soil pH to a value favourable for the growth of improved species (i.e. ≥ 5.5); and drainage, which may be undertaken if necessary. At or near the time of sowing, further fertilizer applications will be made: N (40-100 kg ha^{-1}), P (40-60 kg ha^{-1}) and K (60-120 kg ha^{-1}) - which is especially necessary on peats. The new species sown are most commonly a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), but small amounts of cocksfoot (*Dactylis glomerata*), timothy (*Phleum pratense*) or red fescue (*Festuca rubra*) may also be included. The subject of hill land improvement has been reviewed by Newbould (1974/75, 1976).

3.1 Effects of hill improvement operations

(a) Cultivation

Cultivation of the area to be reseeded is used to reduce competition from indigenous species and to provide a suitable seed bed, but it may not always be carried out due to cost or site limitations - e.g. drainage, slope or access. Where cultivation is performed, it will distribute nutrients and organic matter more evenly within the surface soil.

1. Organic matter

Organic matter and copper:

The strong affinity between Cu and OM has been well reviewed by Stevenson and Ardakani (1972) and Stevenson and Fitch (1981). Copper deficiency is commonly found associated with soils of high OM content (e.g. Pizer *et al.*, 1966). The majority of Cu present in soil solutions is complexed with OM (Sanders, 1982) (up to 99% in calcareous soil (Hodgson *et al.*, 1966)); the amount of complexing increases with increasing pH up to a point at which the complexes break down (McBride and Blasiak, 1979; Sanders and Bloomfield, 1980; Sanders, 1982). Cu complexed with OM of molecular weight <1000 is much more readily available to plants than that complexed with OM of molecular weight >5000 (Mercer and Richmond, 1970), possibly because of weaker linkage or lack of steric hindrance (Petruzzelli and Guidi, 1976).

Organic matter and molybdenum:

A close relationship between soil OM and Mo content has been demonstrated (Karimian and Cox, 1978) which is attributed to the adsorption of the Mo^{5+} cation, formed by reduction of MoO_4^{2-} by OM (Szalay and Szilagyi, 1968). It has recently been observed that Mo(V) can be further reduced to Mo(III) by soil OM (Goodman and Cheshire, 1982)

and this may also have a role in Mo-OM complexation. The effect that the association of Mo and OM has on the availability of Mo to plants is not fully understood because of interactions with other factors such as pH, drainage and micro-organisms.

Sulphur mineralization:

(*Mineralization* is the conversion of complex organic molecules by microorganisms into simpler forms which can be absorbed by plants.)

In humid regions, over 90% of total soil S may be present in organic forms (Haque and Walmsley, 1972; Scott and Anderson, 1976). The C : N : S ratio of soils is remarkably constant - e.g. 140 : 10 : 1.4 (Williams *et al.*, 1960), 100 : 10 : 1.8 (Jones *et al.*, 1972) and 114 : 10 : 1.4 (Lee and Speir, 1979), but the rates of mineralization of N and S differ (Freney *et al.*, 1962; Tabatabai and Bremner, 1972). The magnitude of the difference will vary with the soil (Williams, 1967b; Freney *et al.*, 1971). In the short-term, both faster rates of mineralization of N relative to S (e.g. Williams, 1967b; Swift, 1977) and slower rates (e.g. Tabatabai and Al-Khafaji, 1980) have been observed. In the long term, both N and S appear to be mineralized in the same ratio as they are present in OM (Williams, 1967a). The pattern that S mineralization follows will also vary between soils and seems to be related to the varied nature of decomposing soil OM rather than to any other soil property (Freney and Swaby, 1975).

The rate of mineralization is affected by factors which influence microbial activity.

pH - the addition of lime increases the rate (e.g. Ellett and Hill, 1929; Probert, 1976).

Temperature - increasing temperature increases the rate, and temperatures above 20°C are the most favourable (Williams, 1967b; Lee and Speir, 1979; Kerr, 1981).

OM - both form and amount are significant; the higher the OM content of the soil the more rapid the rate is (Harward *et al.*, 1962). McLaren and Swift (1977) observed that although 75% of the S mineralized came from the C-bonded organic S fraction, it was the HI-reducible organic S that was more important in short-term mineralization, the C-bonded S was converted to HI-reducible S prior to mineralization.

Aeration/water content - as oxygen supply decreases so the rate decreases. Soil water contents <15% and >40% were found to be unfavourable (Williams, 1967b).

Drying and rewetting - when soils are dried and then moistened the rate increases, probably due to the Birch effect (Williams, 1967b; Kerr, 1981), but physico-chemical processes may also be involved. (The *'Birch effect'* is germination of desiccated bacterial spores, and subsequent multiplication of the bacteria, which occurs when a dried soil is rewetted. The young microbial population has a high rate of metabolic activity and enzyme production, leading to rapid rates of mineralization of OM (Birch, 1958)).

The presence of plants - both increasing (Freney and Spencer, 1960; Cowling and Jones, 1972) and decreasing rates (Barrow, 1967; Jones *et al.*, 1972) have been observed.

In some surface soils, ester sulphate (HI-reducible organic S) can account for about 50% of total soil S (Tabatabai and Bremner, 1972b); so it has been proposed that sulphatase enzymes may play an important

role in soil organic S mineralization (Tabatabai and Bremner, 1970), but the observed rates of release of S by mineralization are much lower than those theoretically possible from calculations based on aryl sulphatase activity in the laboratory (Freney and Swaby, 1975). Possible reasons for this discrepancy include: non-ideal conditions in the field, microbial interference, the non-occurrence of specific sulphatase enzymes in the soil (general soil enzymes being responsible for the breakdown of ester-sulphates), aryl sulphatases may not occur in the soil, the sulphate group is in a position inaccessible to the enzymes or the environment in which it is held (e.g. on clays) inhibits enzyme activity (Freney and Swaby, 1975).

It has been observed quite frequently that mineralization alone is insufficient to meet plant S requirements when immobilization and volatilization of soil S are also considered (e.g. Harward *et al.*, 1962; Kerr, 1981) and atmospheric S inputs to soil and plants are important (see page 13).

2. *Microorganisms*

Cultivation and the disturbance of soil OM may stimulate soil microbial activity. Soil microorganisms can affect the availability and solubility of soil trace elements directly by alkylation (chelation), alteration of valency state and the uptake and incorporation of trace elements into the cell; and indirectly by the synthesis of extracellular microbial metabolites, the secondary synthesis of high molecular weight humic substances and via changes in soil pH and Eh (Cataldo and Wildung, 1983).

Microorganisms and copper availability:

Mycorrhizal fungi have been observed to increase the Cu uptake of plants (Lambert *et al.*, 1979; Gildon and Tinker, 1983) and this has been demonstrated to be not merely because of enhanced plant growth due to raised P content of the plant (Gildon and Tinker, 1983). It is probable that Cu is absorbed and translocated by the fungi in a similar manner to P.

Microorganisms and molybdenum availability:

Rhizosphere organisms appear to encourage the plant uptake of Mo. The magnitude of the effect depends on soil type, and the organisms may also cause release of some of the unavailable soil Mo (Cheng and Ouellette, 1973). Microbes may also reduce the amount of Mo available for plant uptake by forming Mo-Cu complexes in their cells (Fleming, 1980).

Microorganisms and sulphur availability:

See S mineralization.

(b) Drainage

The drainage status of a soil has been described as "the most important pedological factor in Scottish soils influencing trace element availability" (Mitchell *et al.*, 1957b), and is said to dramatically affect the trace element content of herbage, particularly Mo, as indicated by the data in Table 1.3 (overleaf).

If a site is particularly poorly drained, waterlogged conditions can develop with associated anaerobic and reducing conditions. Reducing conditions alter soil characteristics in many ways: disruption of soil oxides and OM which causes both the displacement of ions from exchange

Table 1.3: Soil drainage status and herbage copper and molybdenum (from Fleming, 1973).

Soil drainage status	Concentration in ryegrass (mg kg^{-1} DM) Mo	Cu
Moderately free	2.2	11
Imperfect	3.1	11
Imperfect/poor	2.9	12
Poor	3.9	14
Very poor	13.0	11

sites and the development of new surfaces for ion adsorption, so cation/anion balances and mineral equilibria are altered, as are pH and the specific conductance and ionic strength of the soil solution (Ponnamperuma, 1972, 1984). Plant growth and mineral nutrition will therefore be affected; not only because of the altered nutrient availability from the soil and inhibited root extension, but also because of the possible accumulation of toxic substances, such as ethanol in the roots or ethylene, or organic acids, in the soil (Devitt and Francis, 1972; Nambiar, 1975).

In Scottish field conditions it is unlikely that a soil will be continually waterlogged, rather it will be subject to cycles of wetting and drying - reduction and oxidation. On re-oxidation/aeration, the amorphous oxides produced by the reducing conditions will become more crystalline either desorbing metal ions that have been adsorbed under the reducing conditions or incorporating them into the crystal lattice. Rewetting again (further reducing conditions) will cause the oxides (and organic matter) to break down, possibly releasing adsorbed ions. The overall effect of periodic waterlogging in field conditions may therefore be increased metal uptake by plants (Iu *et al.*, 1982).

Soil water and copper:

Cu is not itself subject to oxidation/reduction changes on waterlogging, but its mobility within the soil and hence its availability to plants will be affected by the breakdown of soil oxides and OM (Jarvis, 1981a). Conflicting reports exist as to whether extractable soil Cu increases (e.g. Ng and Bloomfield, 1962; Kubota *et al.*, 1963) or decreases (e.g. Kubota *et al.*, 1963; Basak *et al.*, 1982) on waterlogging. The presence of lime (raised pH) or high OM content will also affect soil extractable Cu content - decreasing it due to the intensification of reducing conditions (e.g. Ng and Bloomfield, 1962; Haldar and Mandal, 1979; Iu *et al.*, 1981; Iu, 1981). A detailed investigation into the effect of waterlogging on the different fractions of Cu in Scottish soils was undertaken by Iu (1981). He discovered that whereas the amounts of soluble, exchangeable and organically bound Cu decreased with waterlogging the amounts of Cu specifically adsorbed or adsorbed onto oxides increased.

Differences have also been reported in the effect that soil waterlogging has on plant uptake of copper - increases (e.g. Adams and Honeysett, 1964) and decreases (e.g. Beckwith *et al.*, 1975) have been observed. Species differences could be partially responsible: Iu *et al.* (1982) found that whereas waterlogging increased Cu uptake by French bean, it decreased it in maize; and Mitchell *et al.* (1957b) observed a 10-fold increase in the extractable Cu content of red clover in poorly drained field conditions, whilst that of PRG was unaffected. Another possible reason for the conflicting observations could be competition for uptake with Zn (Iu *et al.*, 1982).

Soil water and molybdenum:

Poor drainage or waterlogging of a soil leads to dramatic increases in the extractable Mo levels of both the soil (Ng and Bloomfield, 1962) and the total Mo level of herbage growing in it (Mitchell *et al.*, 1957b; Kubota *et al.*, 1963). The rise in herbage Mo levels is due to increased availability of Mo in the soil because of increasing solubility (Kubota *et al.*, 1963), release from soil oxides and OM as they break down (Ng and Bloomfield, 1962); and also to enhanced mass flow and diffusion in the soil solution (Mitchell, 1971).

The role of OM in increasing Mo availability in waterlogged soils is poorly understood; some authors consider it important (e.g. Fleming, 1980) and others do not (e.g. Allaway, 1977). Ng and Bloomfield (1962) observed that reaeration of a flooded soil caused less immobilization of Mo than would have been expected in an organic system and they suggest that OM may have inhibited co-precipitation of Mo with Fe oxides.

Soil water and sulphur:

In waterlogged soils the bacterium *Desulfovibrio* reduces sulphate to sulphide which is either evolved as H_2S gas or forms insoluble metal sulphides with Fe, Zn or Cu (Ponnamperuma, 1972, 1984). Volatile sulphur compounds are also produced in waterlogged soils and dimethyl sulphide may be of more importance than H_2S for the loss of sulphur from the soil (Lovelock *et al.*, 1972).

Neutral and alkaline soils show an immediate decrease in water-soluble sulphate on flooding, while in acid soils the decrease is preceded by an initial increase due to the release of sorbed sulphate from clay and hydrous Fe and Al oxides with increasing pH (Ponnamperuma, 1972).

Williams (1975) reported that although poorly drained soils were richer in sulphate than freely drained ones, because there was less leaching, the total S content of herbage growing on the poorly drained soil was less than that growing on the freely drained soil; however, Reddy *et al.* (1981b) could find no effect of different soil water regimes on plant S content. Scott and Anderson (1976) found no consistent effects of soil drainage conditions on soil organic S contents and attributed any apparent influences of drainage to effects on soil OM rather than organic S specifically.

(c) Liming

Lime is applied to hill soils to reduce the concentration of H^+ ions and the solubility of Al while increasing the availability of Ca and possibly some trace elements (Russell, 1973). The amount of lime that is applied to the soil will depend on its buffering capacity and texture, for example, organic soils have lower optimum pHs for plant growth than mineral soils (Mengel and Kirkby, 1982).

The effect that liming has on trace element availability in the soil will depend on the balance between the effects of decreased H^+ ion concentration, increased Ca^{2+}, increased HCO_3^- and enhanced microbial activity (DeKock and Cheshire, 1968).

Liming and copper availability:

The effect that lime application has on the availability of soil Cu to plants will vary with soil and plant species. Mineralization of soil OM will be stimulated by liming (Ellett and Hill, 1929) and this may lead to a release of Cu; however, adsorption of Cu by the variably charged soil oxides and OM will increase as pH increases (e.g. McLaren and Crawford, 1973b; McLaren *et al.*, 1981), and non-specific adsorption

(although only of minor importance) may increase due to reduced competition as the H^+ concentration alters. Within the pH range 5 to 7 the concentration of Cu in the soil solution does not alter appreciably as, although solution Cu^{2+} concentration decreases, Cu-OM complexation increases (McLaren *et al.*, 1981, 1983a, 1983b; Sanders, 1982; El-Kherbawy and Sanders, 1984).

The initial soil Cu status may modify plant Cu response to liming. On Cu deficient soils, plant Cu concentration may slightly decrease while on Cu adequate soils it may increase or exhibit no change (Burridge *et al.*, 1983). Plant species may also modify the effect of liming on Cu uptake. Mitchell *et al.* (1957b) observed that although the Cu concentration of ryegrass remained constant at 2.9 mg kg^{-1} when liming raised the pH from 6.0 to 6.4, that of red clover increased from 4.7 to 6.8 mg kg^{-1}. Possible damage to plant tissue by $\overset{\text{high}}{\wedge}H^+$ concentrations with consequent impaired ability to absorb Cu, will be reduced as pH increases (Graham, 1981).

Liming and molybdenum availability:

The addition of lime to a soil greatly increases plant Mo uptake (e.g. Mitchell *et al.*, 1957b; Williams and Thornton, 1972) and may be used in some circumstances to eliminate Mo deficiency. This increase in plant available Mo is due to decreased adsorption of Mo brought about by anion exchange of OH^- and $H_2PO_4^-$ for molybdate as pH increases (see page 14), and to the release of Mo from mineralized OM (Ellett and Hill, 1929). The increased concentration of available soil Mo is sufficient to overcome the decreased ability of plants to absorb molybdate from solutions of increased pH (Stout *et al.*, 1951). Lindsay (1972) stated that for each unit increase in pH, the molybdate concentration in the soil solution increases 100-fold (assuming a total Mo concentration of $10^{-7.5}$M at pH 6.5).

In contrast to decreased soil adsorption of Mo, with increased pH, increasing Ca^{2+} concentration leads to increased molybdate adsorption (Barrow, 1972).

Liming and sulphur availability:

The plant availability of soil sulphate increases with liming, provided no leaching occurs (Williams and Steinbergs, 1964; Elkins and Ensminger, 1971). Liming increases the rate of S mineralization (Williams, 1967b) and encourages anion exchange between OH^- and SO_4^{2-} on Al and Fe hydrous oxides (Harward and Reisenauer, 1966); and the higher pHs increase the solubility of molybdate and phosphate compounds, so competition for adsorption sites occurs (Metson and Blakemore, 1978). The relative contribution of these processes will depend upon the soil and environmental conditions; in very acidic soils, sulphate availability may be raised due to increased solubility of sparingly soluble hydroxysulphates with increased pH (Adams and Rawajfih, 1977; Wolt, 1981).

Barrow (1972) has demonstrated increased sulphate adsorption as Ca^{2+} concentration increases, as it does for molybdate.

Liming and phosphorus availability:

The effect that lime has on Cu, Mo and S availability will be influenced by its effect on soil phosphate content. The interactions of lime and phosphate have been reviewed by Haynes (1982). The principal conclusions are summarized here:

- P availability increases as pH increases from 4 to 7;
- in soils with high Al^{3+} content new polymeric-hydroxy-aluminium cations will be formed that adsorb P;
- mineralization of OM will be stimulated, releasing organic P;

- insoluble calcium phosphates precipitate out at high pHs reducing P availability;
- since liming overcomes Al toxicity, the inhibition by Al of P uptake, translocation and utilization will be overcome.

(d) Fertilization

Fertilizers are applied to hill soils to raise the nutrient status to a suitable level for the growth of the introduced species. The three major nutrients - N, P and K - are added and possibly some trace elements; hence, many interactions can occur and the effect of one element on plant uptake of another is difficult to interpret. The effects that fertilization has on the soil include: alteration of soil conditions, such as pH (e.g. $(NH_4)_2SO_4$ acidifies the soil); addition of minor nutrients as impurities in the NPK fertilizers; and alteration in the balance of major to minor nutrients which may affect plant uptake. Plant trace element content may also be altered by effects on the stage and form of plant growth. In particular, a dilution effect (the same amount of trace element distributed in a bigger plant) may result which, together with changes in the botanical composition of a pasture, will reduce the trace element concentration of grazed herbage (Burridge *et al.*, 1983).

Effect of nitrogen on copper uptake:

The difficulty in interpreting (and predicting) the effect that fertilization has on plant trace element content is demonstrated well by the effect of N fertilizer on plant Cu uptake.

On soils of low to deficient Cu status, herbage Cu concentration decreases when N is applied, which may lead to Cu deficient plants (e.g. DeKock and Cheshire, 1968; Chaudhry and Loneragan, 1970).

On soils of adequate Cu status (either naturally or through the addition of copper fertilizer) the application of N increases plant total Cu concentration (DeKock and Cheshire, 1968; Hill *et al.*, 1978), but this is not always associated with an increase in plant Cu concentration as the dilution effect may have occurred (Chaudhry and Loneragan, 1970). Even if an individual plant's Cu concentration does increase, the overall Cu content of the pasture may not rise as the percentage of clover present will be reduced and, on Cu adequate soils, clovers generally contain more Cu than grasses (Reith *et al.*, 1984).

The form of N fertilizer applied may also affect plant Cu content. On Cu adequate soils nitrate sources lead to greater Cu uptake than ammonium sources, NH_4NO_3 occupies an intermediate position (Cheshire *et al.*, 1967; Tills and Alloway, 1981a). Similarly, on Cu deficient soils NH_4^+-N causes a greater reduction in plant Cu status than either NO_3^--N or NH_4NO_3 (Tills and Alloway, 1981a): possible reasons for the decreased Cu uptake are competition for cation adsorption sites on the root surface (Cox and Reisenauer, 1977) (Cheshire *et al.* (1967) in a study of oats on Cu deficient peat suggest that the addition of N may upset the Cu/Fe balance at site of uptake leading to lowered Cu uptake), acidification of the root media (Blair *et al.*, 1970) and disturbed cation/anion balance (Kirkby and Mengel, 1967).

The relationship between Cu and N in plants has already been discussed (page 11). Copper deficiency may lead to a loss of control over N uptake and a possible toxic accumulation of NH_4^+ ions (Cheshire *et al.*, 1982) and affect N metabolism by inhibiting protein synthesis leading to the accumulation of free amino acids (Tills and Alloway, 1981b).

Effect of phosphorus on copper uptake:

The addition of P fertilizer may increase plant Cu uptake (Cheshire *et al.*, 1967), but it is generally found that plant Cu content decreases slightly when P is added (e.g. Singh and Swarup, 1982; Reddy *et al.*, 1981b). The decrease is accounted for both by the dilution effect and the formation of insoluble $Cu_3(PO_4)_2$ which reduces plant available Cu (Dolar and Keeney, 1971).

Interactions between N and P occur; Singh and Swarup (1982) found the adverse effect of P fertilizer on Cu uptake was minimized when N fertilizer was also applied.

Effect of potassium on copper uptake:

Little information is available on the effect of K on plant Cu uptake, but Graham (1979) reports decreased Cu uptake by sunflowers after K fertilization.

Effect of minor elements on copper uptake:

Copper as a fertilizer - Many different forms of Cu have been used as fertilizers on Cu deficient soils - Cu sulphate, mixtures of Cu sulphate and Cu hydroxide, Cu oxide, Cu carbonate, Cu oxychloride, Cu chloride, Cu slag, Cu frits, ammonium based liquid Cu fertilizer, Cu chelates or in NPK compounds; and various methods of application - soil treatments, foliar sprays and seed dressings (Graham and Nambiar, 1981). The method most commonly used if Cu is applied during hill improvement is a surface soil dressing of $CuSO_4$ at the rate of 6 kg Cu ha^{-1} (Mitchell *et al.*, 1957b). This method entails less risk of Cu toxicity to stock than foliar application and lasts longer; residual effects have been observed after 25 years at one site (Burridge *et al.*, 1983), although other workers have detected no residual influences after only

2 years (Evans, 1983); and is equally as effective as the more expensive EDTA-Cu (McLaren and Williams, 1981).

Copper fertilizer has been reported to raise the Cu content of clover and other legumes to a greater extent than that of grasses (Burridge *et al.*, 1983), but the positive interaction of Cu and N fertilizers must be taken into account (see above).

Aluminium - Even at concentrations as low as 0.1 mg kg^{-1} Al, an antagonistic interaction occurs between Cu and Al leading to decreased total Cu uptake, probably because of competition for root adsorption sites (Blevins and Massey, 1959; Hiatt *et al.*, 1963). Liming will help to overcome this antagonism.

Iron - Reports vary as to the effect of Fe on Cu uptake. Cheshire *et al.* (1967) observed the interaction of Cu and Fe to be mutually antagonistic in oats with excessive amounts of one decreasing the uptake of the other, probably because of competition for non-specific uptake sites. However, if excessive iron was applied to plants of low Cu status a synergistic interaction was observed. Dokiya *et al.* (1968) observed that whereas the addition of Fe decreased Cu uptake in rice, for barley it had no effect on Cu uptake.

Manganese - Differing views exist about the effect of Mn on Cu uptake. All possible interactions have been observed - synergistic (Dolar and Keeney, 1971), no effect (Bowen, 1969) and antagonistic (Dokiya *et al.*, 1968).

Molybdenum - Cu and Mo have been observed to exhibit mutual antagonism with the application of one adversely affecting plant uptake of the other (e.g. McKay *et al.*, 1966).

Sulphur - No reference has been found to any effect of S on plant Cu uptake.

Zinc - Both Zn and Cu are absorbed by a similar, possibly identical, mechanism into the plant (Bowen, 1969), so uptake is competitive with Zn addition decreasing Cu uptake - both amount and rate (Chaudhry and Loneragan, 1970) - although the magnitude of this effect will vary with soil conditions and rate of application (Arora and Sekhon, 1982).

Effect of nitrogen on molybdenum uptake:

There is little information available on the effect of N fertilizer on plant Mo uptake, but the form of N appears important. NO_3^--N seems to increase plant need for Mo, however, as Mo is vital for the correct functioning of nitrate reductase it is difficult to be sure of this (Gupta and Lipsett, 1981). When present as NH_4^+, N appears to decrease plant Mo uptake (Williams and Thornton, 1972) although if SO_4^{2-} is the accompanying anion the effect at the NH_4^+ ion may be obscured (Hemingway, 1962). Barshad (1951) observed the effect of N fertilizer, possibly NH_4^+ ions, to cause a delay rather than reduction in plant Mo uptake.

Effect of phosphorus on molybdenum uptake:

The addition of P fertilizer stimulates plant Mo uptake especially on acid soils (Barshad, 1951; Stout *et al.*, 1951; Murphy *et al.*, 1981). Anion exchange occurs between phosphate and molybdate ions on the soil surfaces (Stout *et al.*, 1951), so more Mo is available for absorption; and although P and Mo compete for plant uptake individually, a complex phosphomolybdate anion is formed which is more readily absorbed by the plant than molybdate is (Barshad, 1951).

Reports also exist of P fertilizer reducing plant Mo uptake, but this is most probably due to contamination of the fertilizer with S (Gupta and Cutcliffe, 1968), although alteration in the relative balance of P-displacement of Mo from adsorption sites and competition between P and Mo for plant uptake may be responsible (Gupta and Lipsett, 1981).

Burridge *et al*. (1983) reported that herbage Mo contents were not increased when P was applied together with N or K.

Effect of potassium on molybdenum uptake:

Little information exists about the relationship between K and Mo, but it is believed to be antagonistic (Cheng and Ouellette, 1973). Burridge *et al*. (1983) reported that in the absence of P the addition of K (at two N levels) increased herbage Mo content but the effect may have been due to the Cl^- anion applied with the K^+.

Effect of minor elements on molybdenum uptake:

Aluminium - Fleming (1980) reports the existence of an antagonism.

Iron - An Fe-Mo antagonism exists. The application of Mo decreases the amount of extractable soil Fe and also the Fe content of plants growing in the treated soil because of the formation of an iron molybdenum complex of low solubility in the soil (Gupta and Mehla, 1979 - Subterranean clover).

Magnesium - The interaction is reported to be synergistic (Fleming, 1980).

Manganese - The uptake of Mo and Mn seems to be mutually antagonistic (Cheng and Ouellette, 1973).

Sulphur - As molybdate and sulphate behave so similarly in soil, it is not surprising that the application of one closely affects the uptake of the other. The application of S as SO_4^{2-} has been observed to depress plant Mo uptake (Stout *et al.*, 1951; Reddy *et al.*, 1981a) probably because of direct competition for uptake (Stout *et al.*, 1951). Occasionally a synergistic relationship has been observed depending on soil type (Fleming, 1980); Barshad (1951) observed that the application of S as gypsum increased the availability of Mo and attributed this to the acidifying effect of gypsum decreasing soluble CO_3^- and OH^- ions in the soil, and on competition for uptake.

Molybdenum fertilizer - Mo fertilizer is not commonly applied in the U.K. as soils are normally Mo sufficient and, since Mo is "freely" taken up by plants (Gupta and Lipsett, 1981), the application of Mo will increase the risk of adverse Cu : Mo ratios developing in pastures. If Mo fertilizer is applied it will have a greater effect on plant Mo contents on mineral rather than organic soils, although the addition of N fertilizer may mask the increase due to the dilution effect (Reith *et al.*, 1984).

Effect of nitrogen on sulphur uptake:

The addition of N to a soil decreases plant sulphate concentration probably because of the dilution effect (McLaren, 1976; McPherson *et al.*, 1978). Again, the form of N used is important with urea > NH_4^+ > NO_3^- for both total and organic plant S content, probably because of an effect on protein metabolism (Kirkby, 1978).

The ratio of total N : total S in healthy plants is relatively constant at 14 : 1 for graminaceous species and 17.5 : 1 for leguminous species (Dijkshoorn and Van Wijk, 1967). The range of values for legumes is wider than that for grasses (Whitehead and Jones, 1978).

Effect of phosphorus on sulphur uptake:

P fertilizer stimulates plant SO_4^{2-} uptake (McLaren, 1976) by decreasing soil adsorption of sulphate which leads to increased sulphate availability - the sulphate may be adsorbed lower down the soil profile (Kamprath *et al.*, 1956; Metson and Blakemore, 1978).

As for P and Mo, the effect of P on plant S uptake may be modified by the soil, for example, Reddy *et al.* (1981a) observed the sulphate uptake of subterranean clover to be decreased by P addition.

Effect of potassium on sulphur uptake:

Little information exists about the interaction of K and S, but McLaren (1976) observed a decrease in herbage SO_4^{2-} levels when K fertilizer was applied. He attributed this both to the dilution effect and the antagonistic effect of the accompanying Cl^- ions.

Sulphur fertilizer:

In the U.K. it has seldom been necessary up till now to apply S fertilizer, but it may become necessary in the future. The main reasons for this are:

1. the reduction in the S content of fertilizers following the replacement of $(NH_4)_2SO_4$ by NH_4NO_3 and urea, and of single superphosphate by triple superphosphate;
2. the increases in crop yields and therefore crop uptake, associated with the introduction of improved varieties and greater use of fertilizers; and
3. the reduction in atmospheric pollution (Scott and Munro, 1979; Kerr, 1981).

(e) Species

The indigenous vegetation (dominated by *Agrostis*, *Festuca*, *Molinia*, *Nardus* or *Calluna*, depending on soil type) is replaced by the more productive PRG and WC, possibly with small amounts of timothy, red fescue or cocksfoot, during reseeding.

Species differences in copper uptake:

On soils with adequate Cu, whether naturally or because of Cu fertilizers, legumes contain more Cu than grasses (Mitchell *et al.*, 1957a; Gladstones *et al.*, 1975). On soils of low Cu status the position may be reversed (Mitchell *et al.*, 1957a), this has been accounted for by greater variability in legume Cu content, 1-12 mg kg^{-1}, than grass Cu content, 3-5 mg kg^{-1}, with changing soil Cu status (Mitchell, 1963). More recent work has not shown the order legumes > grasses to be altered by soil Cu status (Beavington and Wright, 1977; Forbes and Gelman, 1981).

Broad leaved herbs are reported to have high mineral contents relative to sown species (e.g. Burridge *et al.*, 1983), but Forbes and Gelman (1981) found that none of 6 herbs they studied was richer in Cu than WC. Table 1.4 gives some values for the concentrations of Cu and Mo that have been found in some herbs and other species and shows the order herbs ≧ legumes > grasses, with regard to Cu concentration.

Within the grasses the order cocksfoot > PRG > timothy is found (Davey, 1957; Forbes and Gelman, 1981). The latter authors observed no highly significant differences between different varieties of cocksfoot, PRG or WC, although others have observed varietal differences, e.g. Reuter *et al.* (1983) for subterranean clover.

Table 1.4: Concentrations of copper and molybdenum in different herbage species. (From Forbes and Gelman, 1981.)

Species	Concentration in dry matter (mg kg^{-1}) Cu	Mo
Lolium perenne (PRG)	3.7	0.8
Dactylis glomerata (Cocksfoot)	5.2	1.3
Trifolium repens (WC)	6.7	1.3
Actillea millefolium (Yarrow)	6.4	0.8
Taraxacum officinale (Dandelion)	5.7	0.6

Possible reasons for species (and varietal) differences in Cu content include: the rate of Cu absorption by roots, better root exploitation/exploration of the soil either because of large roots or more root hairs, modification of the form of available Cu by root exudates, acidification or change in redox potential of the soil by the plant, more efficient retranslocation of Cu from old to young tissue or lower tissue requirements for Cu (Graham, 1981).

Species differences in molybdenum uptake:

Legumes are usually found to contain more Mo than grasses (Fleming, 1973; Forbes and Gelman, 1981), but this may vary with soil Mo content. Among the grasses, cocksfoot has been observed to have a particularly high Mo content (Davey, 1957). Forbes and Gelman (1981) found it to be as high as the Mo content of WC.

Species differences in sulphur uptake:

Grasses have been reported to take up more (Metson, 1979), less (Gilbert and Robson, 1984) or similar (McLaren, 1976) amounts of S compared to legumes. Typical grass total S concentrations are

0.20 - 0.45% (Whitehead, 1972) and significant differences have been shown between *Lolium* cultivars (Smith *et al.*, 1982).

3.2 The effect of season on plant mineral content

The effect of season on plant Cu, Mo and S contents is not affected by reseeding, but is nevertheless a factor that must be considered as herbage Cu, Mo and S contents are found to vary both within and between season. Table 1.5 gives data for a reseeded pasture and shows how the levels can vary (from Evans, 1984).

Table 1.5: The effect of season on the herbage concentration of copper, molybdenum and sulphur in a reseeded pasture.

	Concentration in dry matter		
Sampling date	Cu (mg kg^{-1})	Mo (mg kg^{-1})	S (%)
May 1979	6.3	3.9	0.52
July 1979	4.5	2.3	0.26
Sept 1979	4.0	4.0	0.32
May 1980	2.9	2.0	0.34
July 1980	3.1	2.5	0.30
Sept 1980	3.4	2.7	0.34

(from Evans, 1984)

Seasonal variations in herbage copper content:

The pattern of change of herbage Cu levels with season has not been found to be consistent: no change (e.g. Whitehead and Jones, 1969), decreasing throughout the years (e.g. Evans, 1984), increasing June to October (e.g. Mitchell *et al.*, 1957a), or a marked initial decrease May to July followed by an increase to September (e.g. Davies and Crawshaw, 1978).

Species differences are also observed. Fleming (1965) states that whereas the Cu content of grass declines with maturity, that of clover increases, defoliation frequency will affect how this influences pasture Cu levels.

Seasonal variations in herbage molybdenum content:

Again, reports vary as to the effect that season has on plant Mo content. Most authors have reported increasing plant Mo levels as the season advances from June to October (e.g. Davey, 1957; Davies and Crawshaw, 1978), but no change (e.g. Reddy *et al.*, 1981b), decreases (e.g. Fleming, 1973), initial decreases followed by an increase (e.g. Evans, 1984) or an initial increase followed by a decrease (e.g. Jarvis and Austin, 1983) have also been reported.

Seasonal variations in herbage sulphur content:

Again, reports differ as to the effect that season has on herbage total S content; no variation (e.g. Williams, 1975; Whitehead *et al.*, 1983 for PRG), increases as the season progresses (e.g. Kerr, 1981), decreases as the season progresses (e.g. Whitehead *et al.*, 1983 for WC) and initial declines followed by increases (e.g. Jarvis and Austin, 1983). Species differences have also been noted; a decrease of S with maturity has been reported for alfalfa (Pumphrey and Moore, 1965).

The reason for the existence of conflicting reports about the effect of season on herbage Cu, Mo and S contents is because many factors influence them. For example, soil temperature, soil water content, fertilizer applications, grazing management/frequency of defoliation which affects the stage of maturity of the plant, and the relative species composition of a pasture (Reddy *et al.*, 1981b). The influence of these

factors individually is poorly understood so the combination of several, which may themselves be affected by season is very difficult to interpret, so prediction of the effect that season may have on the occurrence of induced copper deficiency in sheep is also complicated; autumn (Suttle, 1982), August (Davies and Crawshaw, 1978) and late June/early July (Jarvis and Austin, 1983) have all been reported to be the times of highest risk.

A summary of the reported effects that the factors discussed above have on the availability of Cu, Mo and S to plants is presented in Table 1.6.

Table 1.6: Summary table of the reported influences of various factors upon the availability of copper, molybdenum and sulphur to plants.

Factor		Element Cu	Mo	S
pH		↓↑	↑	↑
OM		↓	?	↑
Poor drainage		↓↑	↑	↓?
Season		✓	✓	✓
Other nutrients:	N	↑↓	↓?	↓
	P	↓(↑)	↑	↑
	K	↓?	↓?	↓?
	others	✓	✓	✓
Microorganisms		Mycorrhiza	Rhizosphere	Mineralization

↑ - increase
↓ - decrease
↑↓ - increases and decreases reported
? - literature is inconclusive
✓ - an interaction occurs

NB This table is a generalization, specific interactions will depend on soil type, initial soil nutrient status, plant species, plant part, etc.

4. INDUCED COPPER DEFICIENCY IN SHEEP

4.1 Susceptibility

The susceptibility of a sheep to induced Cu deficiency will vary with genetic, physiological and dietary factors.

(a) Physiology

The efficiency with which a ruminant can absorb Cu decreases rapidly from 80-90% at birth to <10% at weaning and into maturity (Suttle, 1974). This, coupled with the decline in Cu content of ewe's milk as lactation progresses (Ashton *et al.*, 1977), can have a significant effect on induced Cu deficiency as the lambs must obtain a proportion of their Cu requirements from the herbage - which may not be possible (see page 2).

(b) Genetic factors

Different breeds of sheep have differing susceptibilities to induced Cu deficiency apparently because of varying efficiencies of Cu absorption (Wiener *et al.*, 1978). The Scottish Blackface, a predominant breed on Scottish hill farms, is more susceptible to induced Cu deficiency than Cheviots, Welsh mountain sheep or any of their crossbreeds (Wiener and Field, 1970).

(c) Dietary factors

As already discussed (page 3), it is the relative concentrations of Cu, Mo and S in the sheep's diet rather than the absolute concentrations which are important. The exact balance of the three that gives rise to problems is not known, but Miltimore and Mason (1971) in a study with cattle suggest that a herbage Cu : Mo ratio of <2 : 1 can cause induced Cu deficiency.

Several factors may influence the balance of Cu, Mo and S in the sheep's diet:

Pasture improvement - discussed above.

Soil ingestion - Up to 20% of the dry matter intake of a sheep may be soil (MAFF, 1982); the quantity of soil ingested will vary with type of pasture, management factors, soil type, season and weather conditions, but tends to be especially high in the autumn and winter months when grass is in short supply (Thornton, 1983). The availability of dietary Cu to the sheep is reduced by soil ingestion regardless of the soil Mo content - an inclusion of 10% soil in the diet leads to a 50% reduction in Cu availability to sheep (Suttle *et al.*, 1975). The reduction could occur either due to association of dietary Cu with one or more soil colloids (Jarvis and Austin, 1983) or because of interaction of trace elements, notably Fe, in the soil with dietary Cu - heavy metal sulphides may be formed in the rumen which release their sulphide in the acid abomasum where it can complex with Cu, giving unabsorbable CuS (Suttle *et al.*, 1984).

Other trace elements - Dietary Cu may interact with trace elements in the diet other than Mo and S, e.g. Mn and Fe, so reducing its availability (MAFF, 1982).

Type of feed - Supplementary and conserved feeds generally have higher available copper contents than fresh herbage - they release a greater proportion of their Cu than fresh herbage in the digestive track beyond the rumen, the rumen is the site of the Cu x Mo x S antagonism (Suttle, 1983a).

It has been observed that sheep can absorb Cu three-times more efficiently from hay than from summer pasture, and six-times more

efficiently than from autumn pasture (Suttle, 1982). Brassicas also have higher Cu absorbability than fresh herbage, but because they have low copper contents the amount of Cu absorbed will be low (Suttle, 1983b).

An example of the combined effect of these dietary factors is the reduced incidence of swayback after a hard winter (Alloway, 1973), because of increased supplementary feeding and decreased soil ingestion (Lewis, 1975).

4.2 Prevention

There is no cure for swayback so treatment must be based on prevention by administration of Cu which may also raise lamb growth rates (Whitelaw *et al.*, 1977, 1979). The prophylactic approaches that have been taken are reviewed by SAC/SARI (1982) and MAFF (1982) and include - addition of copper fertilizer to pastures, injection of copper compounds (e.g. $CuSO_4$, Cu-amino acid salts, CuCaEDTA) and oral administration of Cu via licks, drenches and drinking water or, more recently, as copper oxide needles (Whitelaw *et al.*, 1980).

5. OBJECTIVES OF STUDY AND OUTLINE OF EXPERIMENTAL WORK

There is information available on the soil-plant relationships of Cu, Mo and S separately, but little is known about the 3 elements together, particularly in the hill situation. Hence the programme of experimental work presented in this thesis was designed to investigate the effect that the various processes undertaken during hill improvement (reviewed above) have on the soil-plant relationships of the 3 elements together. It is hoped that the information discovered, together with other work currently being carried out at HFRO may, at a future date,

act as a basis for selecting hill sites for improvement, and improvement techniques least likely to give rise to induced Cu deficiency in sheep.

An initial screening of 24 soils was performed and 9 were selected to give a range of the main characteristics believed to influence herbage content of Cu, Mo and S. All of the soils were used in a preliminary experiment (Experiment 1) but in subsequent more detailed experiments the number was reduced to 2 or 3. The experiments were mainly conducted in the glasshouse and were designed to investigate the influence of soil type, lime application, soil water status, plant species and fertilizer and copper applications.

CHAPTER II

Materials and Methods

The objective of this study was to try and elucidate the mechanisms underlying differences in mineral concentrations, specifically Cu, Mo and S, between indigenous hill vegetation and sown pasture, following improvement of the soil. For this objective to be realised it was necessary to investigate the individual effects of soil treatments and the interactions between the treatments without the confusing influences of external environmental conditions. Therefore the technique of pot experimentation under standard glasshouse conditions was used rather than field experiments.

The problems of trying to extrapolate from the results of pot experiments to the field because of the nature of the former type of experiments (restricted pot volume, artificial temperature and moisture regimes and lack of competitive influences), are acknowledged. However, relative responses of plant growth and nutrient content to soil amelioration and the principles of the mechanisms underlying these responses should be broadly applicable in both laboratory and field conditions. At the least, the interactions between trace element uptake, application of lime and fertilizers, and soil type and conditions should be applicable to the interpretation of soil and herbage nutrient content determined on samples taken in the field.

1. SOIL COLLECTION AND PREPARATION

(a) Preliminary survey

2 - 3 kg samples of top soil, 0-15 cm, were collected using a stainless steel spade. The samples were oven-dried at about 25°C for approximately one week, passed through a stainless steel sieve (2 mm mesh) and then stored in plastic containers to await analysis.

(b) Bulk samples

The turf and root mat were removed from an area approximately 6 m^2, and 100-250 kg of topsoil, 0-15 cm, was collected. The soil was partially air-dried, and then passed through a coarse sieve (1 cm mesh) to remove large stones. The soil was then completely air-dried and placed in polythene lined plastic dustbins for storage.

Soil 6, the peat, was collected as described above, but once partially air-dried it was shredded before being passed through the coarse sieve and stored moist in a polythene lined plastic dustbin at 4°C.

2. SOIL ANALYSIS

Unless otherwise stated:

- all soil analyses were performed on air-dried soil sieved <2 mm;
- for highly organic soils half the quantity of soil stated was taken;
- for fresh soils in Experiment 6, twice the quantity of soil stated was taken.

2.1 Physical Characteristics

Field capacity

A sintered glass crucible of known weight was taken, the sinter saturated, the crucible reweighed, and three-quarters filled with soil. The crucible was immersed in water to the same depth as the soil for two hours and was then removed and left covered until drainage had ceased. The soil and crucible were dried at 105°C until constant weight was attained, they were then cooled and reweighed and the Field Capacity (FC) calculated (after Allen *et al.*, 1974).

$$\% \text{ Field capacity} = \frac{\text{weight of water in soil at saturation} - \text{weight of saturated sinter (g)} \times 100}{\text{oven-dry weight of soil (g)}}$$

Particle fractionation

50 g of soil were taken and treated with 30% hydrogen peroxide to destroy organic matter. The soil was then heated to evaporate excess moisture prior to dispersion in 100 ml of 5% Calgon. After standing overnight the suspensions were stirred for 5 minutes using a high speed dispersion machine and then diluted to 1 litre in a measuring cylinder and placed in a constant temperature room at 19.5°C. Hydrometer readings were taken at 4 minutes 48 seconds and at 6 hours after dispersion (after Floate, 1965). The calculations performed were:

$$\% \text{ clay, } <2\ \mu m = \frac{\text{Corrected hydrometer reading at 6 h (g l}^{-1}) \times 100}{0.50 \times \% \text{ dry matter of soil (g)}}$$

$$\%(\text{silt} + \text{clay}) = \frac{\text{Corrected hydrometer reading at 4 min 48 s (g l}^{-1}) \times 100}{0.50 \times \% \text{ dry matter of soil (g)}}$$

$$\% \text{ silt, } <20\ \mu m = \%(\text{silt} + \text{clay}) - \% \text{ clay}$$

Hydrometer readings were corrected for differences in temperature from 19.5°C and for the Calgon present.

pH

Dry soil: $2x$ ml of 0.01M calcium chloride were added to x g of soil and the suspension stirred. After standing for 30 minutes the suspension was stirred again and left to stand for a further 30 minutes when the pH of the suspension was determined (after Schofield and Taylor, 1955).

Fresh soil: A slurry was made of the soil using 0.01M $CaCl_2$ and after 30 minutes the pH of slurry was determined.

Moisture percentage

1 g of soil was weighed into a crucible of known weight and dried at 105°C until constant weight was attained. The soil and crucible were cooled and reweighed and the moisture percentage calculated:

$$\% \text{ moisture} = \frac{\text{loss in weight of soil on drying (g) x 100}}{\text{initial weight of soil (g)}}$$

Loss on ignition

The soil and crucible from the moisture percentage determination were placed in a muffle furnace at 500°C for 2 hours, they were cooled and weighed and the loss on ignition percentage calculated.

$$\% \text{ loss on ignition} = \frac{\text{loss in weight of soil on ashing (g) x 100}}{\text{oven-dry weight of soil (g)}}$$

2.2 Chemical Characteristics

Extractable aluminium

5 g of soil were shaken, on a reciprocating shaker, with 50 ml M ammonium acetate (NH_4OAc) solution, pH 4.5, for 1 hour and the resulting suspension filtered (Van Den Hende *et al.*, 1952). The amount of Al in the filtrate was determined by an automated colorimetric technique using alizarin red as the indicator (Lancaster and Balasubraumaniam, 1974, modified by Evans, unpub.).

Extractable calcium, iron, magnesium, manganese and potassium

The concentration of Ca, Fe, Mn and Mg in the NH_4OAc extract prepared for Mo were determined by atomic absorption spectrometry (AAS) and that of K by flame emission spectrometry using a IL 251 atomic absorption spectrometer at 422.7, 248.3, 279.5, 285.2 and 766.5

nm respectively. Solutions and standards were diluted 100x with 0.1% $LaCl_3$ to act as a releasing agent; for Mn 0.1% $SnCl_2$ was also added to ensure all the Mn was in the same oxidation state.

Organic carbon

0.2 g of finely ground soil was digested in 50 ml of acid dichromate reagent (0.4 N $K_2Cr_2O_7$ in H_2SO_4 + H_3PO_4) for one hour under reflux. The excess dichromate was titrated against standard ferrous sulphate (approximately 0.4 N) using diphenylamine indicator in the presence of a white background of barium sulphate (Tinsley, 1950).

Cation exchange capacity

2 g of soil were saturated with 50 ml of a buffered Ba^{2+} solution, this was left overnight and then centrifuged at 3750 g for 30 minutes and the pH of the supernatant measured. The process was repeated with fresh buffer until the pH of the supernatant was within 0.1 pH unit of that of the buffer, at which stage it was judged that equilibrium had been obtained. The excess Ba^{2+} was washed out of the soil using 50 ml deionised water (which was then discarded) and 50 ml 0.025M $MgSO_4$ solution was added and the soil left for two hours for exchange to occur between Mg^{2+} and Ba^{2+}. The suspension was centrifuged and the concentration of Mg^{2+} in the supernatant was determined by EDTA titration (after Bascomb, 1964).

This process was performed over a range of pHs from 2 to 8 using the buffer solutions of Helling *et al.* (1964) and the field CEC, that at the pH of the soil in $MgSO_4$, was calculated (Logan and Floate, pers. comm.).

Extractable copper

20 g of soil were shaken, end-over-end, with 100 ml of 0.05M ethylene diaminetetraacetic acid, pH 7.0 (EDTA) for 90 minutes at 20°C ± 1°C (Viro, 1955). The resulting suspension was centrifuged at 20,000 g for 20 minutes and the concentration of Cu present in the supernatant determined by AAS at 324.7 nm using a IL 251 atomic absorption spectrometer.

Extractable molybdenum

(a) The Mo content of the 24 soils initially screened was determined by T.E. Edmonds, Macaulay Institute of Soil Research, using a differential pulse polarographic technique on a neutral NH_4OAc soil extract (Edmonds, 1982).

(b) 10 g of soil were shaken, end-over-end, with 150 ml NH_4OAc, pH 7.0, for 16 hours and the resulting suspension was centrifuged at 20,000 g for 30 minutes. The amount of Mo in the supernatant was determined by flameless AAS at 313.3 nm, using an IL Fastac II auto-injector with an IL 455 flameless atomizer and an IL 251 atomic absorption spectrometer.

The sequence of operation of the IL Fastac II was: stage 1 - omitted; stage 2 - the cuvette was heated to 170°C and the soil extract injected for 40 seconds; stage 3 - the temperature was raised from 170°C to 750°C over 10 seconds; stage 4 - the temperature was increased from 750°C to 1250°C over 15 seconds; stage 5 - the temperature was instantly raised to 3000°C; and stage 6 - the cuvette was held at 3000°C for about 15 seconds. The extracts were all corrected for the presence of background interference: standard additions were used, spiking molybdenum into a bulked sample of several extracts (Mackenzie, unpub.).

Nitrogen

Total nitrogen: The semimicro-Kjeldahl method was used after Bremner (1960). 0.1 - 0.75 g of finely ground soil (amount dependent upon loss-of-ignition %) were digested in 3 ml concentrated sulphuric acid with one 2 g tablet of potassium sulphate-mercuric oxide catalyst for $2\frac{1}{2}$ hours under reflux. The amount of ammonium-nitrogen in the digest was determined by an automated colorimetric technique using the indophenol blue reaction (Allen *et al.*, 1974).

Nitrate nitrogen: 10 g of fresh soil were shaken on a reciprocating shaker with 50 ml of distilled-deionised water for 1 hour, and the suspension filtered. The filtrate was frozen and the concentration of nitrate present determined at a later date using an Orion model 93-07 nitrate ion electrode (Milham *et al.*, 1970).

Ammonium nitrogen: 10 g of fresh soil were shaken on a reciprocating shaker with 50 ml 2M potassium chloride for 1 hour and the suspension filtered. The filtrate was frozen and the concentration of ammonium present determined at a later date using an Orion model 95-10 ammonia electrode on 20 ml of filtrate to which 2 ml of 0.25 M sodium hydroxide had been added (Banwart *et al.*, 1972).

Extractable phosphorus

The concentration of P in the NH_4OAc extract prepared for Al was determined by an automated colorimetric technique based on the formation of molybdenum blue (Ironside, unpub.).

Extractable sulphate

15 g of soil were shaken end-over-end, with 75 ml 0.016M potassium dihydrogen orthophosphate solution (500 $mg\,l^{-1}$ PO_4^{3-}) for 1 hour.

The suspension was filtered, about 1 g of acid-washed charcoal added to decolourize the extract, and shaking was resumed for a further hour (after Massoumi and Cornfield, 1963). The suspension was then centrifuged at 20,000 g for 30 minutes and the concentration of SO_4^{2-} in the supernatant was determined by an automated turbidimetric technique using barium chloride (after Wall *et al.*, 1980).

3. PLANT ANALYSIS

Copper

0.1 g of dried, milled herbage was digested in 1 ml concentrated HNO_3 and evaporated to dryness; a further 1 ml concentrated HNO_3 plus 1 ml 30% H_2O_2 were added and the digest again evaporated to dryness. The residue was dissolved in 2.5 ml 5% HNO_3 and the concentration of Cu present determined by AAS at 324.7 nm using a IL 251 atomic absorption spectrometer.

Molybdenum

The digest prepared for the Cu determination was diluted 10 x with distilled water and the amount of Mo present determined by flameless AAS at 313.3 nm using an IL Fastac II autoinjector (injection time 5 seconds) with a IL 455 flameless atomizer and an IL 251 absorption spectrometer. The sequence of operation of the IL Fastac II was as for the soil extracts but a 5 second injection time was used, not a 40 second one, and standards were prepared in 5% HNO_3.

Nitrogen

0.02 g of dried milled herbage was digested in a 1.2 ml of a sulphuric acid-hydrogen peroxide mixture (350 ml 30% H_2O_2 + 0.21 g

Se powder + 7 g $LiSO_4$ + 210 ml 98% H_2SO_4) and diluted to 10 ml (Parkinson and Allen, 1975). The amount of nitrogen present was determined by an automated colorimetric technique using the indophenol blue reaction (Allen *et al.*, 1974).

Sulphur

0.3 g of dried milled herbage was ground with 1.7 g dried cellulose powder in a Glendevon M260 ball mill and the mixture compressed into a disc using a hydraulic press. The disc was inserted into a Phillips PW 1212 X-ray fluorescence spectrometer and the amount of S present determined (Evans, 1970).

Calcium, Phosphorus and Potassium

The concentrations of Ca, P and K present were determined by X-ray fluorescence spectrometry using the disc prepared for S determination.

4. THE EXPERIMENTS

4.1 Design of the Experiments

The results of the 6 experiments that are reported in this thesis will be considered in relation to the different processes undertaken during hill pasture improvement, rather than as a set of individual experiments. For example, discussion about the effect of liming comes from a consideration of the results of Experiments 1, 2 and 4. Therefore the design of all the individual experiments are summarised here and in Table 2.1.

Table 2.1: Summary table of experimental designs.

Experiment No.	Soils used	No. of lime levels	SPECIAL EXPERIMENTAL TREATMENTS					Species grown	No. of harvests	No. of replicates	Timing of experiment
			Special lime application technique[1]	Fertilizer additions (kg ha^{-1}) (other than basal NPK) N	P	Cu	Water treatments FC soil maintained at	Pre-treatment			
1A	1,2,3,4,5 6,7,8,9	3							PRG	3	4	Nov. 1982-Feb. 1983
1B	2,4,8	5							PRG	3 (destructive)	4	
2A	2,4,8	3	✓						PRG	2	4	Jan. 1983-Apr. 1983
2B	2,4,8	3							PRG & WC	2	4	
3	2,8	1		3 forms - NO_3^-, NH_4^+, urea 3 levels - 0,40,80					PRG	1	4	Mar. 1983-May. 1983
4	2,8	2			3 levels - 24,48,96				PRG & WC	2	4	Jul. 1983-Sep. 1983
5	2,8	1				2 levels - 0,10			PRG & WC	2	4	Sep. 1983-Nov. 1983
6A	2,8	2					60% + waterlogged		*Soil incubation*		3	Oct. 1983-Apr. 1984
6B	2,8	1					60% + 100%	3-dry moist waterlogged	PRG	1	3	

[1]Lime was applied to top 30% (by weight) of soil in pot as one treatment, the other treatment was as for all other experiments - the lime was applied to all the soil in the pot.

Experiment 1: Soil x lime trial

Basic design:

1A 9 soils x 3 pH x 4 reps (PRG; 3 sequential harvests)

1B 3 soils x 5 pH x 4 reps (PRG; 3 destructive harvests)

PRG was grown in nine soils (numbers 1 to 9) at three pHs (lime levels 0, 2 and 4) (Part A); three of the soils (Nos 2, 4 and 8) were studied in more detail at two additional pHs (lime levels 1 and 3) (Part B). The amounts of lime to apply to the soils to give a range of pH from natural (3.0 - 4.9) to 6.5 were determined from a preliminary lime requirement experiment, where the pH of each soil was measured 48 hours after the addition of seven levels of lime. A 1 : 1 mixture of $MgCO_3$: $CaCO_3$ was added to all soils in this and subsequent experiments, as evidence exists that for at least one of the soils studied magnesium, rather than calcium limestone should be applied in the field to achieve maximum response to liming (Rangeley, pers. comm.). Details of the lime additions are given in Appendix 1.1, Table A1.1.

Each treatment was replicated four times and pots were arranged as four completely randomized blocks; an additional pot of each treatment was prepared which was used for preliminary soil analysis. Three harvests were taken on days 35, 60 and 90; the herbage growing on three soils in Experiment 1B was harvested destructively, and that on the other soils in Experiment 1A sequentially.

At the start of the experiment, basal NPK fertilizers were applied to each pot at rates equivalent to 60 kg N ha^{-1}, 48 kg P ha^{-1} and 60 kg K ha^{-1}. After the first harvest a further N application equivalent to 30 kg N ha^{-1} was made, and after the second an application equivalent to 45 kg N ha^{-1}. See Appendix 1.1 for details.

Experiment 2: Soil x methods and rates of lime application x species trial

Basic design

2A 3 soils x 3 pH x 2 species x 4 reps (2 sequential harvests)

2B 3 soils x 3 pH x 2 methods of application x 4 reps (PRG; 2 sequential harvests)

PRG and WC were grown in three soils (Nos 2, 4 and 8) at three pHs; the lime was either applied to all the soil, or to the top 30% by weight of the soil in the pot (top 25% by volume) - application methods A and S respectively. The amounts of lime to apply to the soils to give pHs of 4.5, 5.5 and 6.5 (lime levels 1, 2 and 3 respectively) when application method A was used, were determined from Experiment 1; details are given in Appendix 1.2, Table A1.2. Each treatment was replicated four times and the pots were arranged as four completely randomized blocks. Two sequential harvests were taken on days 40 and 70.

At the start of the experiment, basal NPK fertilizers were applied to each pot at rates equivalent to 60 kg N ha^{-1}, 48 kg P ha^{-1} and 60 kg K ha^{-1}. At germination, 20 ml of a suspension of *Rhizobia* [approximately 1 : 1 FA6/ECA : P3/ECA (Skinner, 1973)] at a concentration of $4 \times 10^6 - 1 \times 10^7$ cells ml^{-1} was sprayed onto the soil surface of each pot After the first harvest an N application equivalent to 30 kg N ha^{-1} was made to each pot (details in Appendix 1.2).

Experiment 3: Soil x nitrogen form x nitrogen level trial

Basic design

2 soils x 3 N-levels x 3 forms of N x 4 reps (PRG; 1 destructive harvest)

PRG was grown in two soils (Nos 2 and 8) which had been limed to pH 5.5. The effect of form and level of nitrogen fertilizer on herbage growth and nutrient uptake was investigated using nitrate-nitrogen (as $Ca(NO_3)_2$), ammonium-nitrogen (as NH_4HCO_3) and urea (NH_2CONH_2) at three levels of application - 0, 1 and 2, equivalent to 0, 40 and 80 kg N ha^{-1}, respectively. At the start of the experiment, basal P and K fertilizer was applied to each pot at rates equivalent to 48 kg P ha^{-1} and 60 kg K ha^{-1}. Details of the amount of fertilizer added are given in Appendix 1.3. Each treatment was replicated four times and the pots arranged as two completely randomized blocks - two replicates per block. (For nitrogen level 0, where form could not be varied, 8 not 12 pots were set up; statistical analyses of the experiment was performed by introducing a factor called 'control' - the presence or absence of nitrogen fertilizer.) One destructive harvest was taken on day 42.

Experiment 4: Soil x lime x phosphorus x species trial

Basic design

2 soils x 2 pH x 3 P levels x 2 species x 4 reps (2 sequential harvests)

PRG and WC were grown in two soils (Nos 2 and 8) and the effect of variations in the levels of lime and phosphate fertilizer on herbage growth and nutrient uptake were investigated. Two levels of lime to give pHs of 5.0 and 6.5 (lime levels 1 and 2) and 3 levels of P (1, 2 and 3) equivalent to P applications of 24, 48 and 96 kg ha^{-1}, respectively, were used. N and K were both applied at rates equivalent to 60 kg ha^{-1} at the start of the experiment. See Appendix 1.4 for full details of fertilizer additions made.

Each treatment was replicated four times and the pots were arranged as four completely randomized blocks; an additional pot of each treatment was prepared which was used for preliminary soil analysis. Two harvests were taken, on days 36 and 52 with an application of 40 kg N ha^{-1} being made to each pot after the first harvest.

Experiment 5: Copper fertilizer trial

Basic design

2 soils x 2 Cu-levels x 2 species x 4 reps (2 sequential harvests)

PRG and WC were grown in two soils (Nos 2 and 8) which had been limed to pH 5.5. Two levels of copper fertilizer were applied (as CuO), 0 and 1 equivalent to 0 and 10 kg Cu ha^{-1}, respectively. Each treatment was replicated four times and the pots were arranged in two completely randomized blocks - two replicates per block; an additional pot of each treatment was prepared which was used for a preliminary soil analysis. Two harvests were taken after 30 and 50 days for the grass, and after 38 and 50 days for the clover.

At the start of the experiment, basal NPK fertilizers were applied to each pot at rates equivalent to 60 kg N ha^{-1}, 48 kg P ha^{-1} and 60 kg K ha^{-1}. After the first harvest a further N application equivalent to 40 kg N ha^{-1} was made. Full details of all fertilizer additions are given in Appendix 1.5.

Experiment 6: Varying moisture regimes

Basic design

6A Soil incubation - 2 soils x 2 pH x 2 H_2O regimes x 3 reps.

6B Pot experiment - 2 soils x 2 H_2O regimes x 3 H_2O pre-treatments x 3 reps (PRG; 1 destructive harvest)

· *Experiment 6A: Soil incubation*

Two soils (Nos 2 and 8) were placed in covered plastic buckets and incubated in a constant temperature growth room at 20°C for 3 months. The soils were left either at their natural pH or limed to pH 6.0, lime levels 0 and 1 respectively, and were either kept moist at approximately 60% of FC (treatment 1) or waterlogged with 2.5 cm standing water on the soil surface (treatment 2). The soils were maintained at the appropriate water contents by weekly additions of deionised water. Three replicates of each treatment were set up. Soil samples for immediate chemical analysis were removed on days 0, 3, 10, 24, 53 and 94 using a plastic soil corer.

· *Experiment 6B: Pot experiment*

Soil from the limed treatments in Experiment 6A was used in a pot experiment together with soil that had been stored dry during the incubation period and to which an equivalent quantity of lime was then added. There were thus three pretreatments of dry (1), moist (2) and waterlogged (3) soils, respectively. The waterlogged soil had the standing water removed and was allowed to dry out slightly for six weeks between Experiments 6A and 6B.

PRG and WC were grown in the pretreated soils which were kept at moisture contents of approximately 60% (1) and 90-100% (2) of FC by watering twice daily with deionised water. The treatments were replicated three times and the pots were arranged in three completely randomized blocks. NPK fertilizer additions equivalent to 60 kg N ha^{-1}, 48 kg P ha^{-1} and 60 kg K ha^{-1} were made to each pot at the start of the experiment. One destructive harvest was taken after 36 days for the grass and after 43 days for the clover. Full details of all fertilizer additions for Experiments 6A and 6B are given in Appendix 1.6.

4.2 Techniques Used in the Pot Experiments

Preparation of the soil

Table 2.2: Amount of stored[1] soil used to fill 10 cm diameter plant pot.

Soil No.	1	2	3	4	5	6	7	8	9
Wt soil (g)	470	420	440	420	400	220	370	270	420

[1]All soils except No. 6 were stored air-dry. Soil 6 (a peat) was stored moist at 4°C.

All pots were set up individually using the following techniques. The pre-determined amount of soil (see Table 2.2) was weighed into a plastic bag and lime and fertilizer in powder form were added according to treatment (see Appendix I); were possible 'AnalaR' chemicals were used. The bag was twisted shut and shaken vigorously to evenly distribute the chemicals throughout the soil. Once the soil dust had settled the bag was opened and the fertilizer amendments that were in solution were added. The bag was again closed and shaken vigorously, both to lay the soil dust and to complete mixing of the chemicals with the soil.

The treated soil was placed in a 10 cm diameter plastic plant pot which was gently tapped to help to pack the soil. The pot was left for 10 days before planting to allow the soil to settle whilst attaining 70% of field capacity (FC). The amount of water to add to each soil had previously been determined (account was taken of the moisture content of the stored soil) and regular additions of deionised water were made to each pot, both from the surface and into the containing saucer until the calculated amount had been applied.

The top layer of soil was removed from each pot and the seeds evenly distributed on the exposed soil surface. A small sample (5-10 g) of the soil that had been removed was retained and dried for use in soil pH determinations, the rest was used to cover the seeds. The seeds sown were either 40 seeds of perennial ryegrass (*Lolium perenne*) cultivar S23 (PRG) placed 1 cm below the soil surface or 30 seeds of white clover (*Trifolium repens*) cultivar New Zealand Grasslands Huia (WC) placed 0.5 cm below the soil surface. To minimize water loss prior to germination the soil surface was covered with an inverted plastic petri dish.

Maintenance

All experiments were conducted in the glasshouses (north-south orientated) at HFRO, Bush Estate, Penicuik. When necessary mercury vapour lamps were used to extend the daylength to 16 hours, and supplementary heating or roof vents were used to maintain temperatures of *c.* 20°C (range 15-23°C).

The soil was kept at approximately 70% FC (except in Experiment 6B) by daily surface watering with deionized water. Twice a week all pots were weighed and the soil returned accurately to 70% FC.

Harvesting

Harvests were taken at the intervals detailed for each experiment.

Sequential harvests: PRG was cut 1 cm above soil level. WC plants had all leaves removed except the newest unexpanded one. The herbage was placed in small aluminium foil trays and dried at 80°C for 16 hours. After the harvest a small dressing of N in solution was applied to each pot (see experimental descriptions for details).

A small soil sample (3-8 g) for use in pH determinations was removed from each pot using a plastic soil corer (about 7 cm deep); the hole was filled with a small clear plastic test tube.

Destructive harvests: PRG leaves were cut at soil level but the bottom 1 cm (stubble) was collected and dried separately. For WC, leaves were collected separately from the stolons.

The soil was left in the pots for 2 days to dry out slightly before being removed and placed in plastic trays in an oven at *c.* 25°C to complete drying, before being stored for use in analysis (pH measurement and chemical determinations).

5. STATISTICAL ANALYSES AND DATA PRESENTATION

All pot experiments were arranged as randomized blocks, replicated 4 times, except for Experiment 6 which had only 3 replicates.

Statistical analyses (analysis of variance, correlation, etc) were carried out on the Edinburgh Multi-Access Computer System using the Mark 4.04 release of Genstat V [copyright 1984, Lawes Agricultural Trust (Rothamsted Experimental Station)] as described in the Genstat manual.

For each analysis of variance, a preliminary analysis was performed on the experimental data, and residual values were plotted against the fitted values. The plots were studied to determine whether the initial analysis was adequate or not, that is whether obvious outliers should be excluded and treated as missing values by Genstat, or whether all the data should be subjected to a logarithmic transformation. If raw data values were excluded for any treatment, the treatment mean is marked ·· in tables and is that given by Genstat. That is, it includes the value generated by Genstat to replace the excluded outlier.

In the results section (Chapter III), unless otherwise stated: (1) only main effects, and occasionally first order, interactions are

presented; and (2) all increases or decreases mentioned were significant generally at $p < 0.001$ (***) but occasionally at $p < 0.01$ (**) or $p < 0.005$ (*). If an increase or decrease is mentioned that was not significant it is termed 'apparent' or 'a trend'.

Graphs were plotted using 'Easygraph' version 2.17 (copyright 1983, Edinburgh Regional Computer Centre).

CHAPTER III

Results

1. SELECTION OF SOILS

A preliminary survey of twenty-four hill soils was undertaken in order to select a smaller number of soils for use in experimental work. The twenty-four soils were collected from eleven sites (Figure 3.1); at each site two small soil samples were collected, one from an improved area and the other from an adjacent unimproved area[1]. The sites were selected to cover a wide range of different soil physical and chemical characteristics, particularly those of interest - Cu, Mo and S content. For example, site 2 was selected as the soil was believed to have a high Mo content, sites 10 and 11 were known to have given rise to swayback in sheep, the soil from site 1 was believed to have a high Al content and the soil at site 6 was a peat.

The soil samples were analysed for the following physical and chemical characteristics - % dry matter, pH, % C, extractable Al, extractable Cu, extractable Mo, extractable PO_4^{3-}-P and extractable SO_4^{2-}-S. The results are shown in Table 3.1 and Appendix II. From these results, soils 1A (Carron Valley), 3A (Glensaugh, Birnie), 4B (Glensaugh, West Finella), 5A (Hartwood), 6A (House o' Muir), 7A (Lephinmore), 9A (Sourhope, Brown Earth), 10A (Sourhope, Peaty Podzol) and 11A (South Slipperfield) were chosen for further investigation. The soils were then renumbered 1 to 9, respectively (these soil numbers are used throughout the thesis).

The selection of the nine soils was carried out by grouping the twenty-four soils into three sets: >4 mg extractable Cu kg^{-1} soil, 1.2-4 mg kg^{-1} and <1.2 mg kg^{-1} (Mackenzie, 1974). From each set three

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[1]From site 2, Dumfriesshire, four samples were collected, all from areas which had been improved. The site was not a hill site but the soil was believed to have a high Mo content, although the analysis did not confirm this.

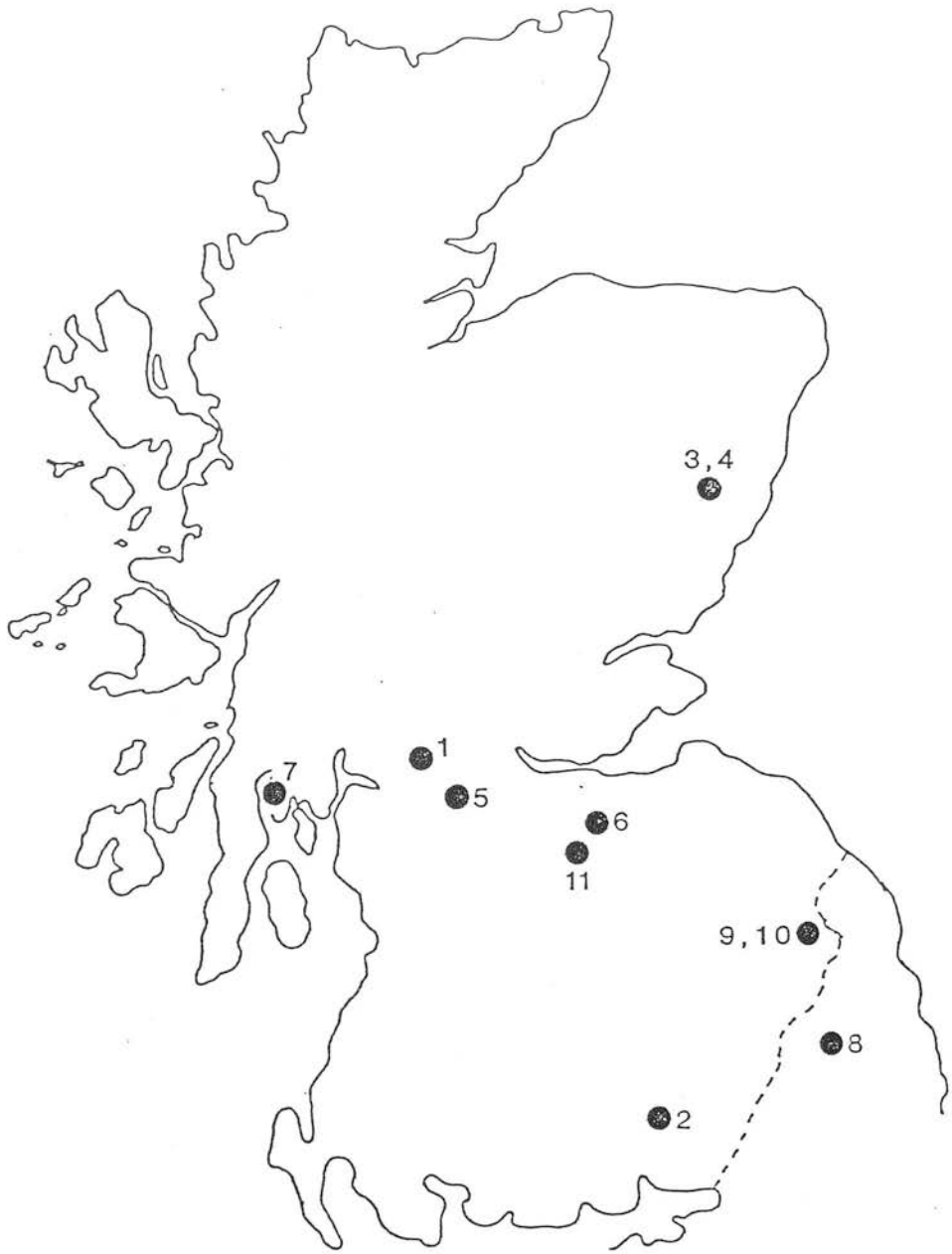

Figure 3.1: Location of the sites where soil was sampled in the preliminary survey.

Table 3.1: Description of the twenty-four soils initially screened.

Site No.†	Site location	Soil type (Scottish classification)	pH	Extractable nutrients (mg kg^{-1} oven dry soil) Cu	Mo	SO_4^{2-}-S
1A	Carron Valley	Peaty gley	3.7	4.4	0.006	28.0
1B	Carron Valley	Brown forest soil	5.2	3.4	0.004	3.2
2a	Gillrigg	Brown forest soil	5.7	1.5	0.024	3.7
2b	Parkgate	Brown forest soil	5.7	3.3	0.011	4.5
2c	Gillrigg	Alluvial/Peat	6.1	5.1	0.025	2.4
2d	Parkgate	Alluvial	5.2	1.9	0.043	5.1
3A	Glensaugh-Birnie	Cultivated brown forest soil	3.5	5.8	0.021	47.0
3B	Glensaugh-Birnie	Cultivated brown forest soil	4.7	2.6	0.095	9.4
4A	Glensaugh-West Finella	Peaty podzol on thin stony drift	3.2	2.4	0.014	14.0
4B	Glensaugh-West Finella	Humus iron podzol on deep till	4.2	0.9	0.305	4.8
5A	Hartwood	Non-calcareous gley	3.3	2.1	0.069	8.7
5B	Hartwood	Non-calcareous gley	5.0	3.1	0.016	3.8
6A	House O'Muir	Brown forest soil	3.6	2.6	0.014	16.0
6B	House O'Muir	Brown forest soil	5.2	1.8	0.027	1.9
7A [2]	Lephinmore	Peat	4.0	4.5	<0.004	26.0
7B	Lephinmore	Peat	4.9	8.3	<0.004	24.0
8A	Redesdale [3]	Peaty gley	5.4	3.0	0.016	18.0
8B	Redesdale	Peaty gley	4.5	1.4	0.013	2.6
9A	Sourhope I	Brown forest soil, low base status	3.5	2.5	0.009	2.0
9B	Sourhope I	Skeletal soil	6.2	2.4	0.033	<0.03
10A	Sourhope II	Peaty podzol	3.2	1.0	0.044	14.0
10B	Sourhope II	Non-calcareous gley	5.7	3.1	0.039	8.2
11A	South Slipperfield	Brown forest soil	4.8	0.8	0.016	4.7
11B	South Slipperfield	Brown forest soil	5.0	1.3	0.026	1.0
	Standard error		*0.02*	*0.14*	[4]	*2.28*

† A - indigenously vegetated site
B - improved site

All samples from site 2 (Dumfriesshire) were from improved areas.

[2] The analyses for the peat were performed on air-dry samples (as for all the soils); and are not strictly comparable to the other values presented.

[3] Soil type 'T' as defined in 'The Soils of Redesdale Experimental Husbandry Farm, Northumberland', MAFF.

[4] The Mo analyses were performed by the Macaulay Institute for Soil Research on single samples so no standard error is available; see Edmonds (1982) for an indication of the precision of the method.

soils were chosen such that the subset covered a wide range of the other measured characteristics. The subsets were 1, 2 and 6; 4, 5 and 7; 3, 8 and 9 - high, moderate and low copper groups, respectively. Finally, from within each group one soil was chosen to be studied in more detail - soil numbers 2 (Glensaugh, Birnie), 4 (Hartwood) and 8 (Sourhope, Peaty Podzol); these 3 soils themselves gave a wide range of the measured characteristics.

Bulk samples of the nine soils were collected for use in pot experiments and samples of the soils were analysed for: pH, CEC, % clay, % LOI, % C, % total N and extractable Cu, Mo, SO_4^{2-}-S, Ca, PO_4^{3-}-P, K, Al, Mn, Fe and Mg. The results are shown in Table 3.2.

The values for the measured characteristics of a soil from a particular site are not identical for the initial sample (24 soils) and the bulk sample (9 soils). This is due to several reasons, including: seasonal influences - the twenty-four small samples were collected in November, whilst the nine bulk samples were taken in July/August; sampling technique - the small sample consisted of several bulked smaller samples taken from the site, whilst the bulk sample was all collected from one pit; and the sample preparation - the small samples were dried in an oven at <25°C, whereas the bulk samples were air-dried in a glasshouse. Generally, however, for a given variable the order of ranking of the nine soils has not altered although the absolute values may have been.

Table 3.2: Description of the nine soils used for the experimental work.

Soil No.	Site[1]	SOIL CHARACTERISTICS																
		pH	CEC (meq $100\,g^{-1}$)	Clay (%)	Dry matter (%)	Loss on ignition (%)	Organic carbon (%)	Total N (mg kg^{-1} oven-dry soil)	Extractable nutrients (mg kg^{-1} oven-dry soil)								
									Cu	Mo	SO_4^{2-}-S	Ca	PO_4^{3-}-P	K	Al	Mn	Fe	Mg
1	Carron Valley	3.9	14	25	94.0	10.5	3.4	0.22	1.7	0.023	42	520	2.8	105	1050	20.5	8.5	230
2	Glensaugh-Birnie	3.7	13	22	92.5	17.5	7.6	0.42	4.0	0.042	30	95	1.7	155	900	6.5	14.0	95
3	Glensaugh-West Finella	4.8	10	19	97.0	10.0	3.9	0.34	0.7	0.036	9	1690	3.1	105	200	29.0	5.0	110
4	Hartwood	3.5	12	28	97.5	15.0	6.4	0.34	2.1	0.032	16	210	1.1	65	350	5.5	37.0	105
5	House o' Muir	3.9	11	35	90.0	20.0	8.3	0.79	1.6	0.028	48	115	7.3	210	1100	60.0	10.0	125
6[2]	Lephinmore	3.2	8	-	38.5	83.0	42.0	2.40	43.0	0.150	66	820	2.5	540	280	20.5	115	1040
7	Sourhope I	3.5	10	24	96.5	21.0	9.7	0.79	1.2	0.038	18	360	6.5	450	320	40.0	18.5	230
8	Sourhope II	3.5	17	20	90.0	41.5	21.0	1.00	0.6	0.049	18	165	4.3	360	510	3.5	15.0	175
9	South Slipperfield	4.4	9	27	94.0	15.0	6.2	0.57	0.8	0.038	17	790	3.8	620	210	20.0	6.5	155
	[3]*Standard error*	*0.02*	*4.0*[4]	*0.8*	*0.1*	*0.4*	*0.2*	*0.008*	*0.05*	*0.0032*	*2*	*19*	*0.31*	*13*	*13*	*0.3*	*0.5*	*22*

[1] For details of location, see Table 3.1 and Appendix II.

[2] For soil number 6, the Lephinmore peat analyses were performed on the damp soil.

[3] Standard errors, excluding soil 6, the peat.

[4] CEC standard error is the mean standard error of the individual standard errors for the 9 soils.

2. RESULTS OF EXPERIMENTS

The results of the six experiments that were undertaken for this thesis are reported in the following section. In all experiments statistical analysis revealed that the majority of treatment main effects and most first, second and third order interactions (if present) were significant at $p < 0.05$, $p < 0.01$ or $p < 0.001$ (i.e. *, ** and *** respectively); so, generally, only main effects and the interactions which produced the largest responses are described.

For each experiment the results are presented in the order:

(a) soil data - pH, extractable Cu, Mo and SO_4^{2-}-S concentrations together with Ca, N and P concentrations if these were measured;

(b) herbage data - DM production, Cu, Mo and S concentrations, and Ca, N or P concentrations for those experiments in which these nutrients were studied.

The data is generally presented graphically and the standard error (s.e.) of the mean is indicated by a bar. The primary data (treatment) means is given in Appendix III, unless tables of data are given in the text (NB for explanation of ¨ see page 65).

2.1 Experiment 1: (Parts A and B) *An investigation into the effect of liming on the content of copper, molybdenum and sulphur in perennial ryegrass grown in nine Scottish hill soils.*

The basic design was:

1A 9 soils (1-9) x 3 pH (lime levels L0, L2, L4) x 4 replicates growing PRG with 3 sequential harvests on days 35, 60, 90.

1B 3 soils (2, 4, 8) x 5 pH (lime levels L0, L1, L2, L3, L4) x 4 replicates growing PRG with 3 destructive harvests on days 35, 60 and 90. (Full details are given in Chapter II, Section 4.1).

The data for soil pH and plant DM production are plotted against time. Data for all other variables are plotted against the soil pH at the harvest under consideration. This latter approach was taken because pH was considered to be a more realistic measure of treatment differences than lime level, since not only were the pHs of the different soils at the same lime levels non-identical (especially at lime level 0) but also different amounts of lime had been added to the different soils at the same lime level (see Table A1.1).

Primary data for the experiment are given in Appendix 3.1. The values for herbage variables for soils 2, 4 and 8 at lime levels 0, 2 and 4 for the first and second harvest in Part B are not always identical to those in Part A. This anomaly arises because treatments in Part B were harvested destructively whilst those in Part A were harvested sequentially; hence different herbage samples were analysed for the same treatment in the two parts of the experiment.

Analyses of variance of the results showed that in Part A all herbage parameters at each of the three harvests and the soil parameters at the conclusion of the experiment had very highly significant ($p < 0.001$) soil and lime main effects and soil x lime interactions. The results for Part B showed that soil pH, soil Cu, Mo and S contents and herbage dry matter production, Mo and S contents to have very highly significant ($p < 0.001$) soil and lime main effects and soil x lime interactions throughout the experiment. However, for herbage Cu this was only the case at the final harvest. Very highly significant ($p < 0.001$) soil, and soil x lime effects were also found for herbage Cu in Part B at the second harvest but the lime effect was only highly significant ($p < 0.01$), and at the first harvest only soil had an effect - very highly significant ($p < 0.001$).

(a) Soil analysis

pH - Part A

There was little variation in the soil pH of each treatment at the four sampling dates (the start of the experiment and each of the three harvests), so mean values for the four soil samples are presented in Table 3.3 (individual means are given in Table A3.1).

Table 3.3: Soil pHs for soils 1 to 9 to which two lime additions were made and samples taken on four occaisons - days 0, 35, 60 and 90.

Soil	pH		
	[1] L0	L2	L4
1	4.1	5.0	7.3
2	3.7	5.1	7.4
3	4.9	5.1	7.2
4	3.4	4.7	7.4
5	3.9	4.9	7.3
6	3.3	4.7	7.3
7	3.6	4.9	7.4
8	3.5	4.9	7.6
9	4.4	4.7	7.1

s.e. = 0.01

[1] L0, L2, L4: lime levels 0, 2, 4

The range of natural soil pHs (unlimed, i.e. lime level 0) was 3.3 - 4.9; the two highest values were found in soils 3 and 9. The former soil was known to have been improved and it is thought that lime may have been spread at some time at the latter site, therefore only small lime additions were needed at lime level 2 (and the resulting soil pH was close to that at lime level 0 (4.9 and 5.1 for soil 3, and 4.4 and 4.7 for soil 9).

pH - Part B

Changes in soil pH with lime were again, generally, small (see Figure 3.2), although for a few treatments lime did have a small effect (this was also found in Part A), e.g. soil 2 lime level 2. Full results are presented in Table A3.2.

The amount of lime to add to a soil at each lime level had been calculated from a preliminary lime requirement trial, and whereas for lime levels 1 and 2 the actual soil pHs were reasonably close to the intended pHs this was not so for lime levels 3 and, particularly, 4 (see Table 3.4).

Table 3.4: Comparison of pH achieved and pH intended for the four lime additions used in Experiment 1.

Lime level	*pH*	
	Intended	Achieved[1] (± s.e.)
1	4.25	4.10 ± 0.006
2	5.00	4.87 ± 0.004
3	5.75	5.97 ± 0.006
4	6.50	7.31 ± 0.004

[1]Grand mean for each lime level.

A possible reason why all the soils were considerably overlimed at lime level 4 is that at such high pHs the buffering capacity of a soil is much reduced, so very small increments in lime addition will have a large effect on pH, whereas at lower pHs, where the soil is better buffered, similar sized increments will have a much smaller influence on soil pH. The amounts of lime to add to soils in subsequent experiments were calculated from lime requirement curves derived from the results of this experiment.

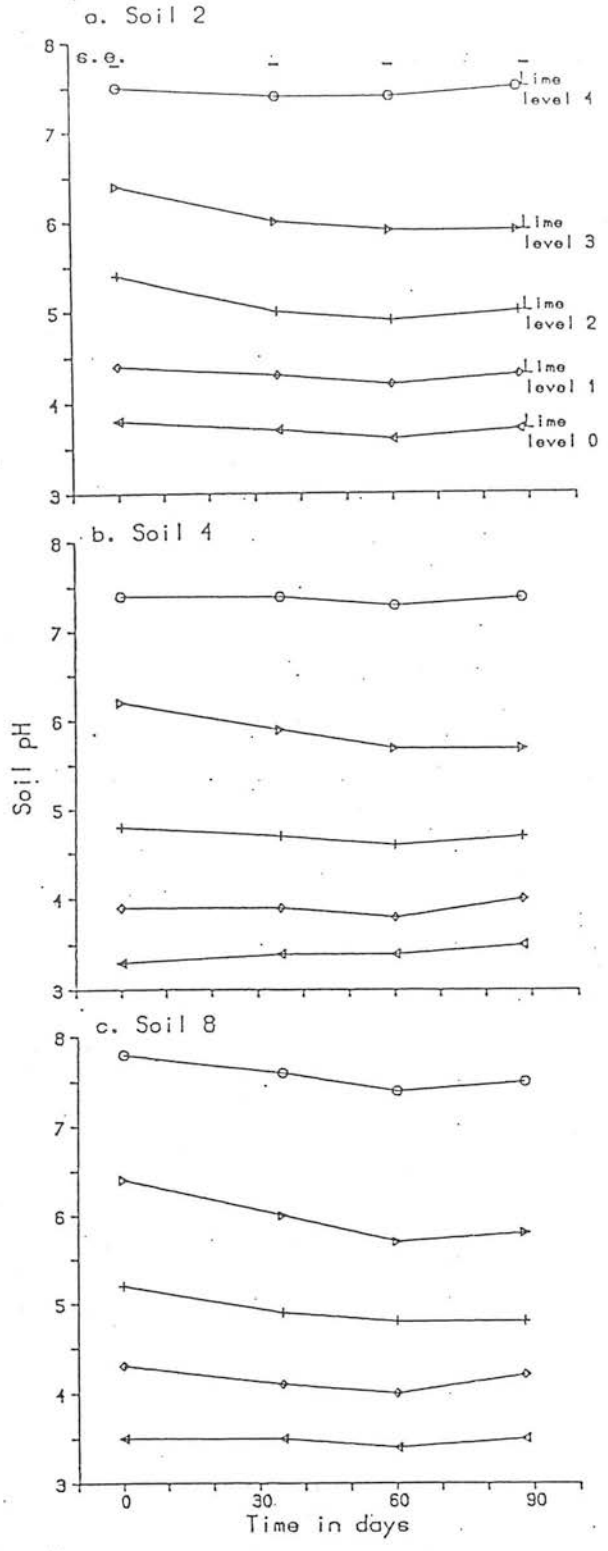

Figure 3.2: pH of soils 2, 4 and 8 in which PRG was grown at five lime levels.

Extractable copper - Part A

There were large differences between soils in extractable Cu concentrations which reflected the initial soil Cu content (*cf.* Table 3.2), and these differences were larger than the within-soil differences due to lime addition (Figure 3.3 and Table A3.3). The effect of liming on soil Cu content was not consistent but most soils showed a small decrease in Cu concentration between lime levels 0 and 2 (i.e. pH ≃3.5 to pH ≃5.0), followed by an increase to level 4, to a higher concentration than that in the unlimed soil.

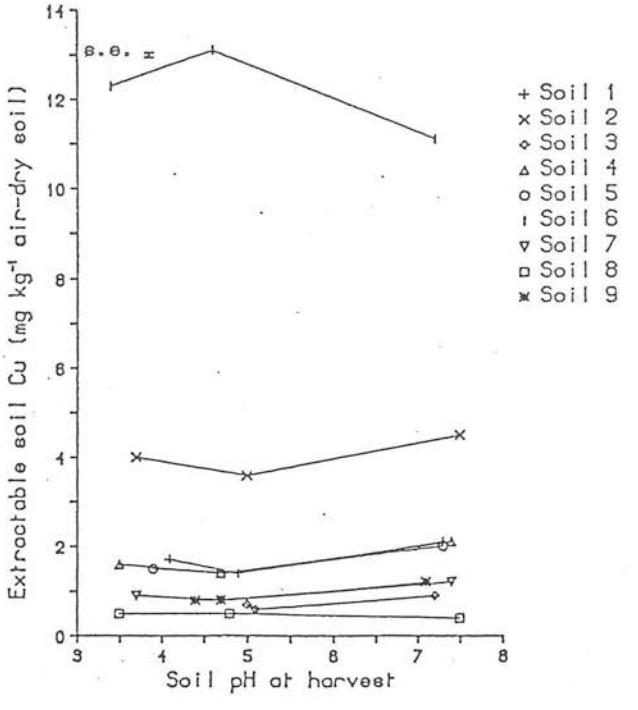

Figure 3.3: Extractable Cu (mg kg^{-1} air-dry soil) in soils 1 to 9 to which lime had been added and in which PRG was grown for 90 days.

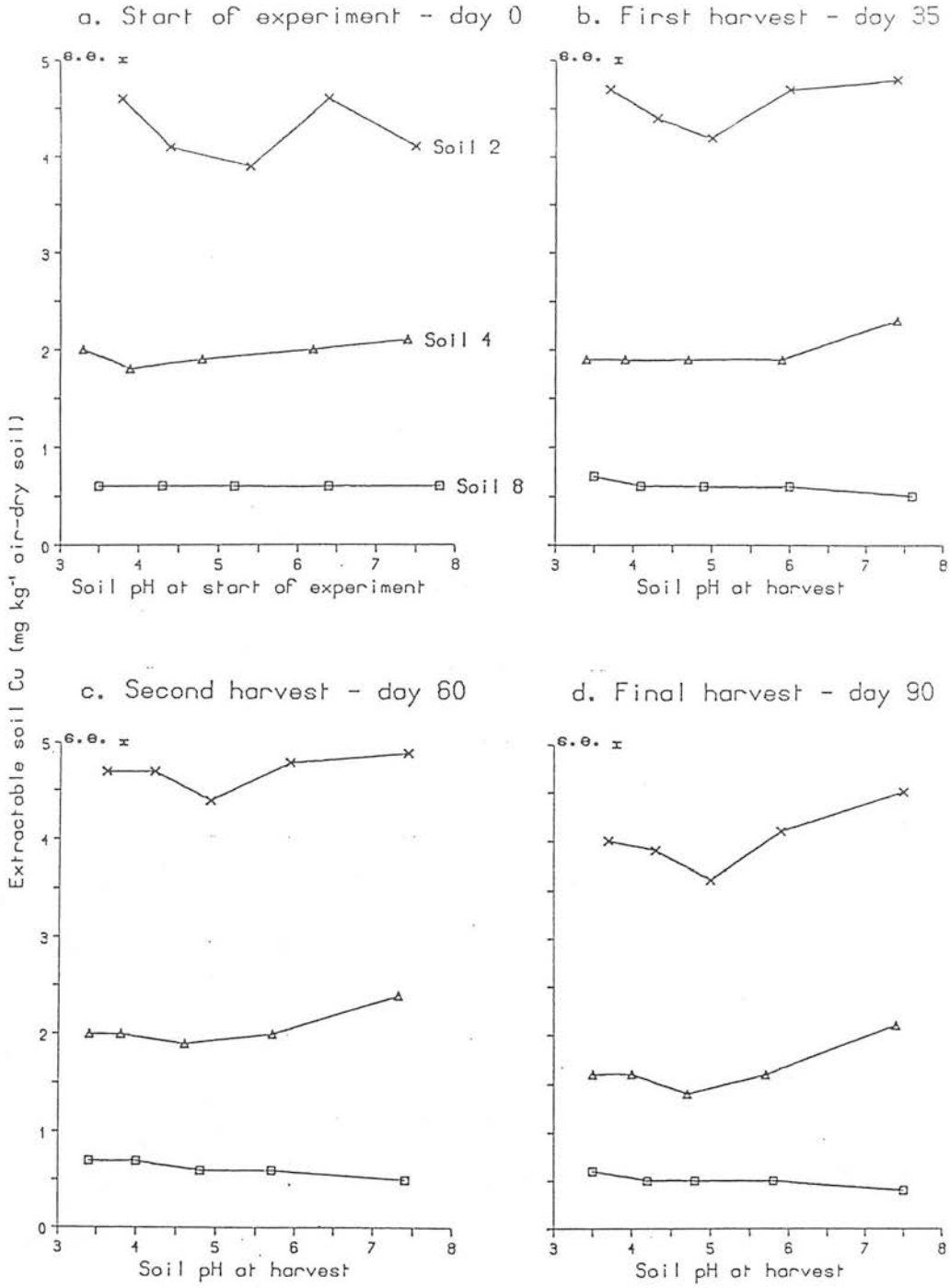

Figure 3.4: Extractable Cu (mg kg^{-1} air-dry soil) in soils 2, 4 and 8 to which lime had been added and in which PRG was grown.

Extractable copper - Part B

Differences between soil were again bigger than within-soil differences due to liming. Changes in Cu content with time were largest in soil 2, the soil with the highest extractable Cu content (Figure 3.4) (4-5 mg kg^{-1} DM). The changes took the pattern of an increase between the start of the experiment and the first harvest, followed by a smaller (not always significant) increase between the first and second harvests and a large decrease at the third harvest - the magnitude of the changes varied with lime level.

The same basic pattern was followed in soil 4 but the magnitude of the changes was smaller and all the increases, apart from the change at lime level 4, between the start of the experiment and the first harvest were not significant. For soil 8, the soil with the lowest extractable Cu content, all changes were not significant apart from that at lime level 1 between the second and third harvests; although the trend did seem to be a decrease in soil Cu with time (Figure 3.4 and Table A3.4).

Extractable molybdenum - Part A

There were large differences in extractable soil Mo both between soils and within soils between lime levels. All soils, except for soil 4, showed overall increased Mo contents with lime addition (L0 *cf.* L4) at the outset of the experiment (Figure 3.5a and Table A3.5). At the final harvest (Figure 3.5b) soils 1, 2, 5, 6, 7 and 8 still showed increased overall Mo content whilst for soils 3 and 9 (the two requiring the least lime amelioration) lime had ceased to have an effect; and for soil 4 the addition of lime apparently caused decreased soil Mo contents. However, all treatments in all soils (except soil 8 L0 and L2) contained more Mo than at the start of the experiment, although this was not always significant.

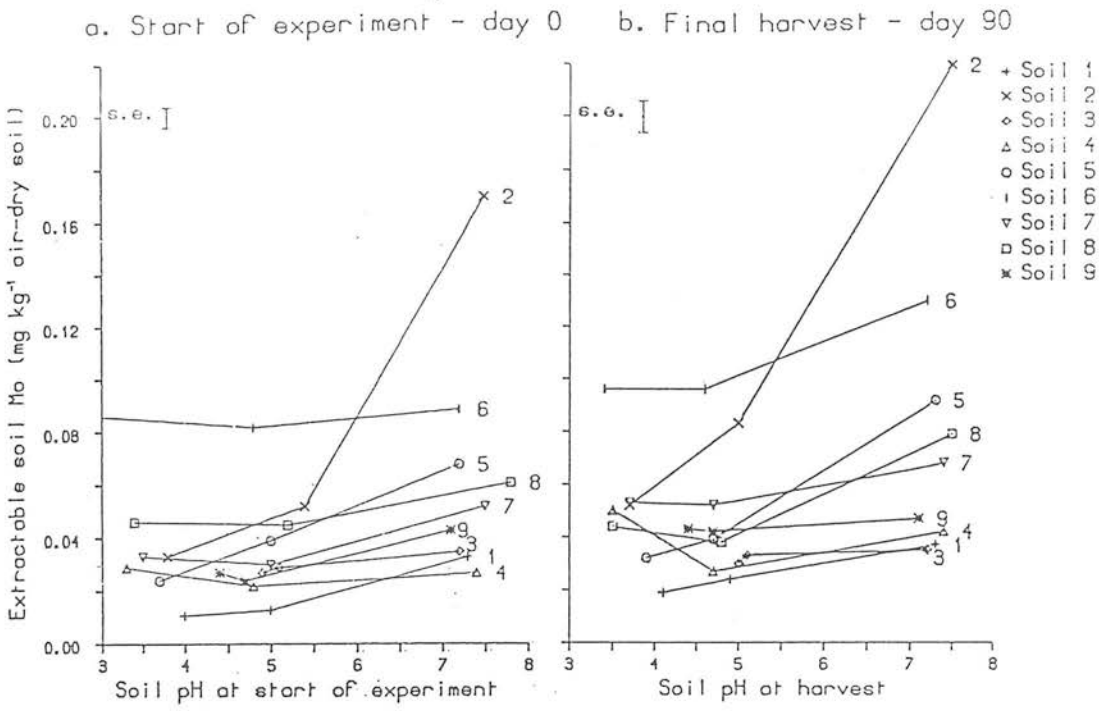

Figure 3.5: Extractable Mo (mg kg^{-1} air-dry soil) in soils 1 to 9 to which lime had been added and in which PRG was grown for 90 days.

For soils 2, 5, 6 and 8 the relative contents of soil Mo at the final harvest for the different lime levels were greater than those at the start of the experiment, especially at lime level 4, i.e. the magnitude of the effect of liming had increased. For soils 1, 3, 7 and 9 the magnitude of the effect of liming had decreased.

Extractable molybdenum - Part B

The three special soils exhibited the range of responses of soil Mo to liming found in Part A (Figure 3.6 and Table A3.6).

Soil 2 showed increased Mo content with the addition of lime, particularly at lime level 4 (it gave the biggest response of the nine soils). There were no effects of time on the Mo content at lime levels 0, 1, 2 and 3 except for a temporary decrease in content at the first

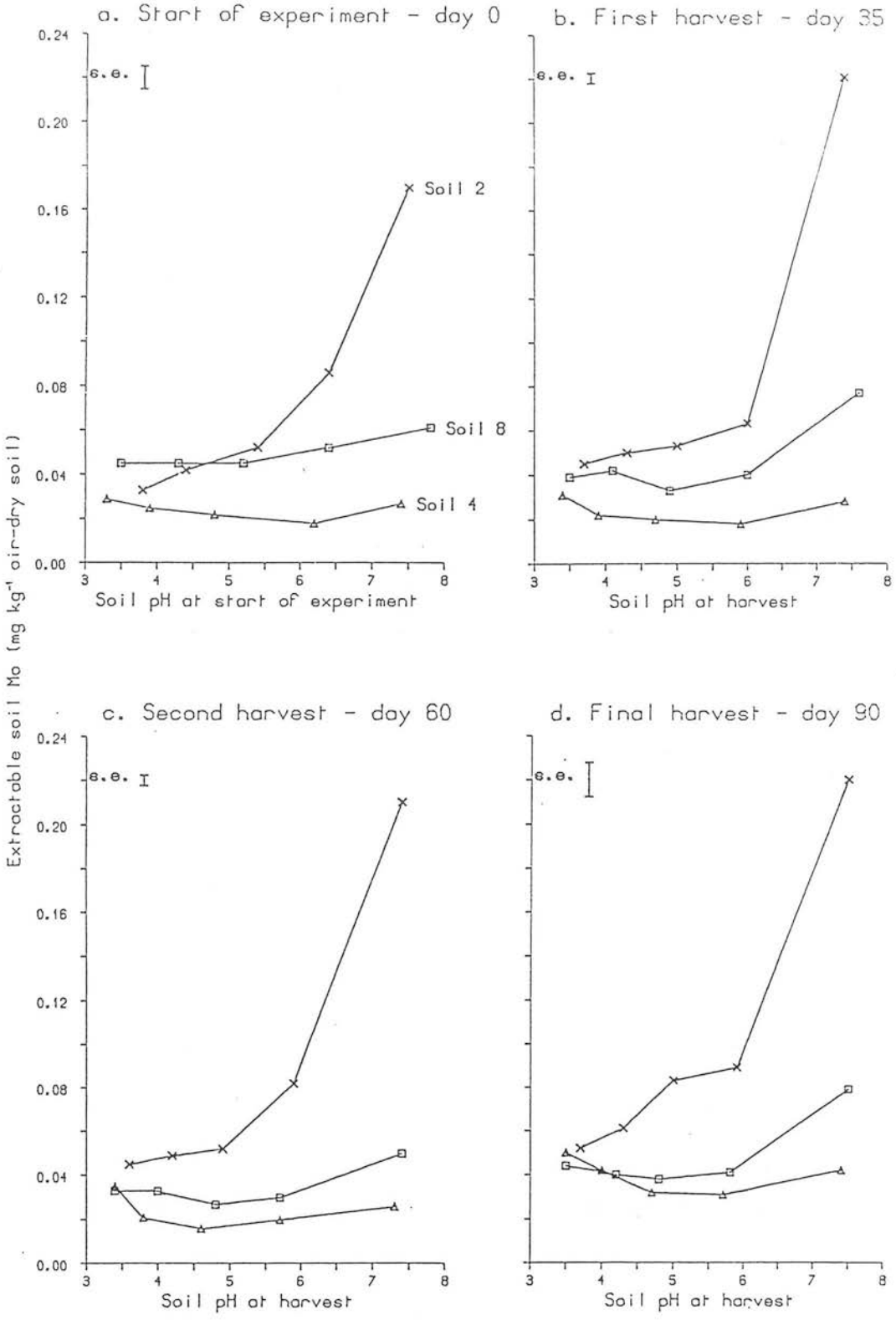

Figure 3.6: Extractable Mo (mg kg^{-1} air-dry soil) in soils 2, 4 and 8 to which lime had been added and in which PRG was grown.

harvest for lime level 3 and an increase at the final harvest for lime level 2; lime level 4 had reached its maximum Mo content (approximately four times the content of the unlimed soil) by the first harvest. This suggests that although time does apparently have an effect on soil Mo content (see Part A), it does not affect soil 2.

Soil 4 showed no significant effect of lime addition on overall (L0 *cf.* L4) soil Mo contents, except for a small decrease at the second harvest, in spite of apparently significant differences in soil Mo contents between lime levels on two occasions (Figure 3.6). Although there was apparently a trend for extractable soil Mo to increase with time it was not significant.

Soil 8, like soil 4, showed no significant effect of lime on Mo at the start of the experiment. At each of the harvests there was a significant overall increase in extractable soil Mo content (L0 *cf.* L4), although it was a lot smaller than the increase observed in soil 2, and all the increase generally occurred between levels 3 and 4. The levels of soil Mo changed with time only at lime level 4 where they increased between the start of the experiment and the first harvest, and the second and third harvests and decreased between the first and second harvests.

Although from Part A it appeared that soil extractable Mo increased with time, from Part B it can be seen that this effect was very variable both with soil and lime level, seeming to occur most frequently at lime level 4.

Extractable sulphate - Part A

The application of lime caused an initial overall (L0 *cf.* L4) increase in soil extractable SO_4^{2-}-S in all soils, except soil 7 (Figure 3.7a). Only soils 1 and 2 had a significant increase of SO_4^{2-}-S contents between lime levels 0 and 2; soil 5 showed an initial decrease and the content in all other soils was not affected by the first lime addition.

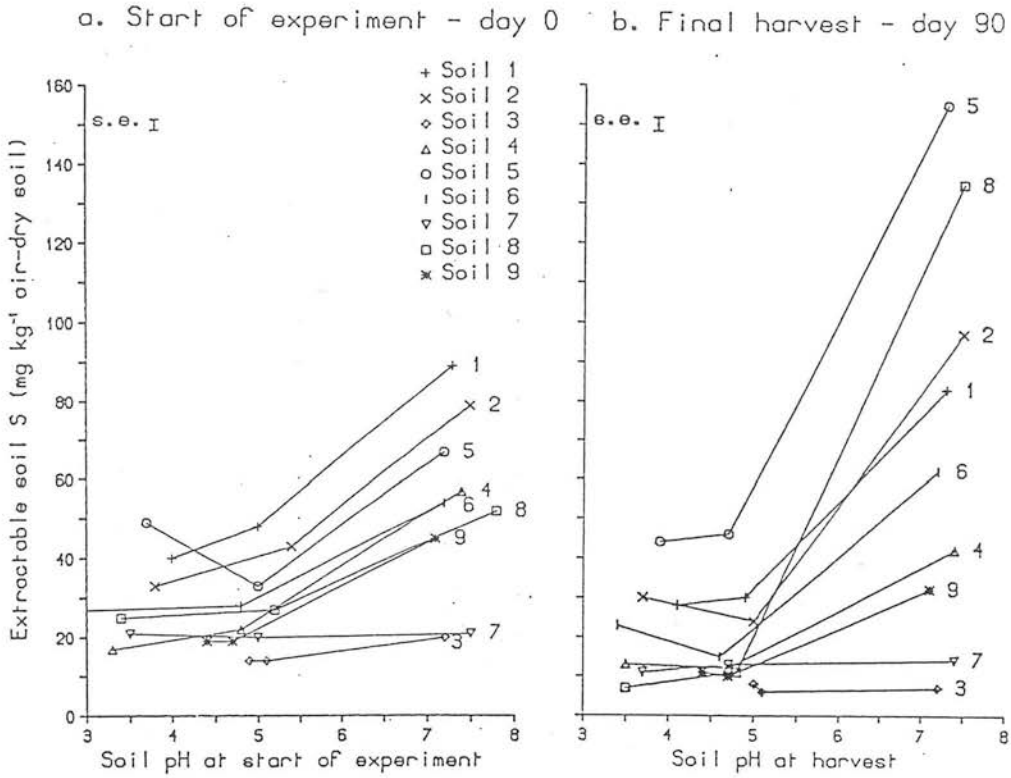

Figure 3.7: Extractable SO_4^{2-}-S (mg kg^{-1} air-dry soil) in soils 1 to 9 to which lime had been added and in which PRG was grown for 90 days.

At the final harvest all soils, apart from soils 3 and 7, showed an overall increase in SO_4^{2-}S contents with lime addition and for these seven soils, apart from soil 6, all the change occurred between lime levels 2 and 4. (Soil 6 exhibited a decrease in SO_4^{2-}-S content between L0 and L2 followed by an increase from L2 to L 4.) (Figure 3.7b)

There was no difference in soil SO_4^{2-}-S content between the two sampling dates in the unlimed treatments for soils 2, 3, 4, 5 and 6 and also soil 1 at lime level 4. The other four unlimed soils (1, 7 8, 9) exhibited decreased SO_4^{2-}-S contents as did all soils at lime level 2, except soil 5 which showed an increase; soils 3, 4, 7 and 9 also showed a decrease at lime level 4. However, the largest differences that were observed were the increased SO_4^{2-}-S contents in soils 2, 5, 6 and 8 at lime level 4 (Table A3.7).

Extractable sulphate - Part B

For soil 2 the addition of lime led to an overall (L0 *cf.* L4) increase in soil extractable SO_4^{2-}-S. The first increment of lime (L0 to L1) led to no significant change in lime level between the start of the experiment and the first harvest, and to decreases between the first and second, and second and third harvests; all other increments caused increased soil SO_4^{2-}-S content, the size of the increase itself increasing as more lime was applied (Figure 3.8).

Soil 4 also exhibited increased overall SO_4^{2-}-S contents as the amount of lime applied increased, and again the magnitude of the increase itself increased at the highest lime levels.

Soil 8 also showed increased overal SO_4^{2-}-S contents as the amount of lime applied increased, and again the magnitude of the increase was larger at the highest lime levels. For the last two harvests a significant increase was observed only between lime levels 3 and 4 whilst for the first harvest smaller but still significant increases occurred between levels 1 and 2, and 2 and 3, and initially there was a decrease between levels 2 and 3.

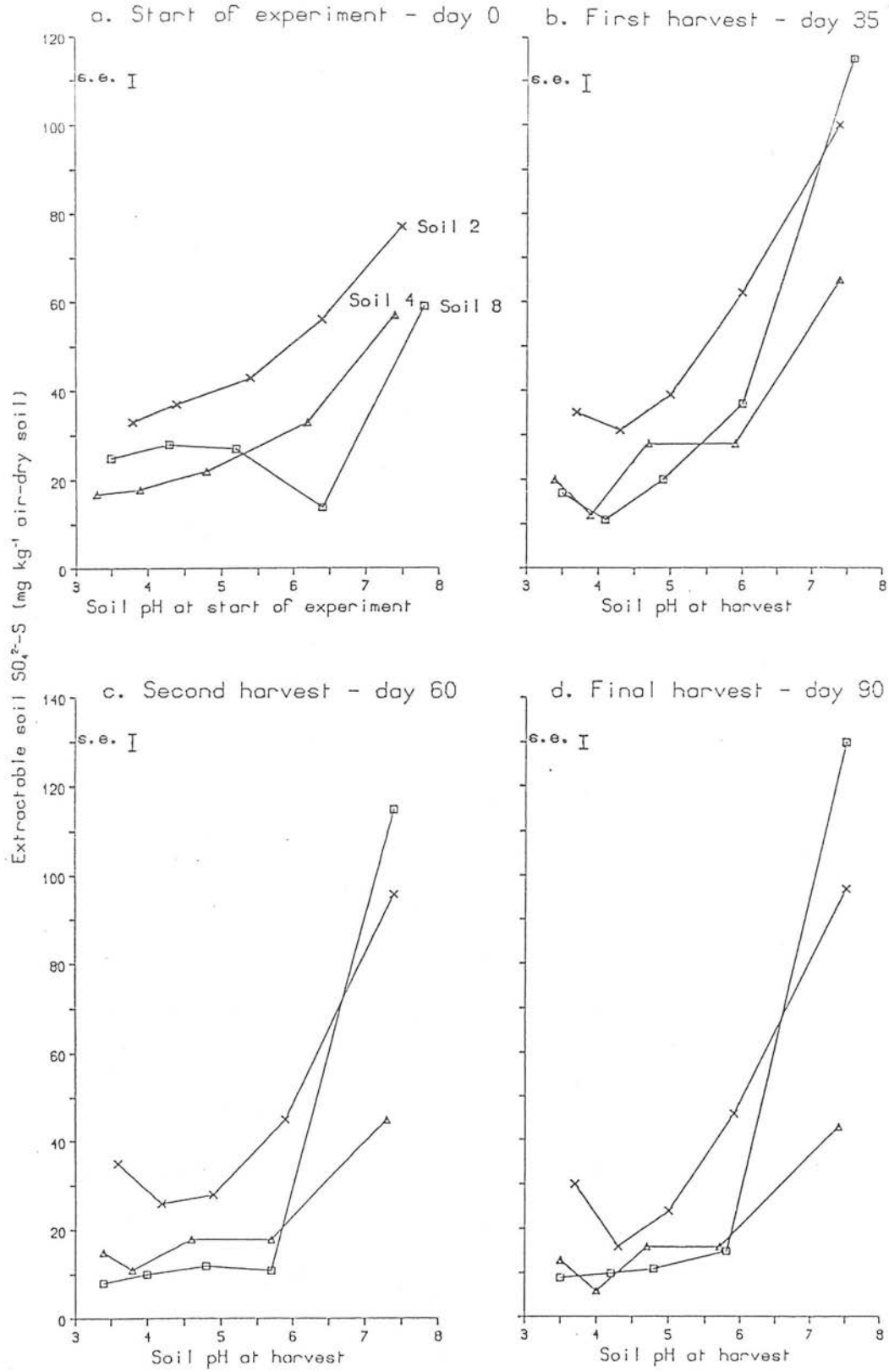

Figure 3.8: Extractable SO_4^{2-}- (mg kg^{-1} air-dry soil) in soils 2, 4 and 8 to which lime had been added and in which PRG was grown.

The change in extractable soil SO_4^{2-}-S between harvests was inconsistent, and all increases had occurred by the first harvest (L4 in all three soils, and L3 in soil 8) (Table A3.8).

(b) Herbage analysis

Dry matter production - Part A

There were large variations in DM production both between the nine soils and within a soil at the different lime levels (Figure 3.9). The range of DM production throughout the experiment was 0.04 - 2.03 g PRG herbage pot^{-1}. The lowest production at all harvests occurred in soil 8 at lime level 4, where the treatment had been considerably over-limed, and the highest in soil 7 (lime level 2 for the first harvest and lime level 4 for the second and third harvests) (Table A3.9).

The overall trend throughout the experiment was for the amount of production to increase as lime was applied and growing conditions improved although this varied with soil, and was less marked for the first harvest at lime level 4, because of the overliming and consequent poor growth, but by the second harvest the adverse effects of the lime had been overcome in all soils, except soil 8. Thus, apart from soil 8, all soils had increased production throughout the experiment (first to third harvests) at lime level 4; although in only two soils (2 and 7) did an increase in production also occur between the second and third harvests, soils 3, 4 and 6 had decreased production during this period and all other soils (1, 5, 8 and 9) had unaltered production (Figure 3.9).

The unlimed treatments (lime level 0) generally had higher production at the second harvest compared to the first (Figure 3.9) (this applied to all soils except soil 7 for which there was no effect and soil 3

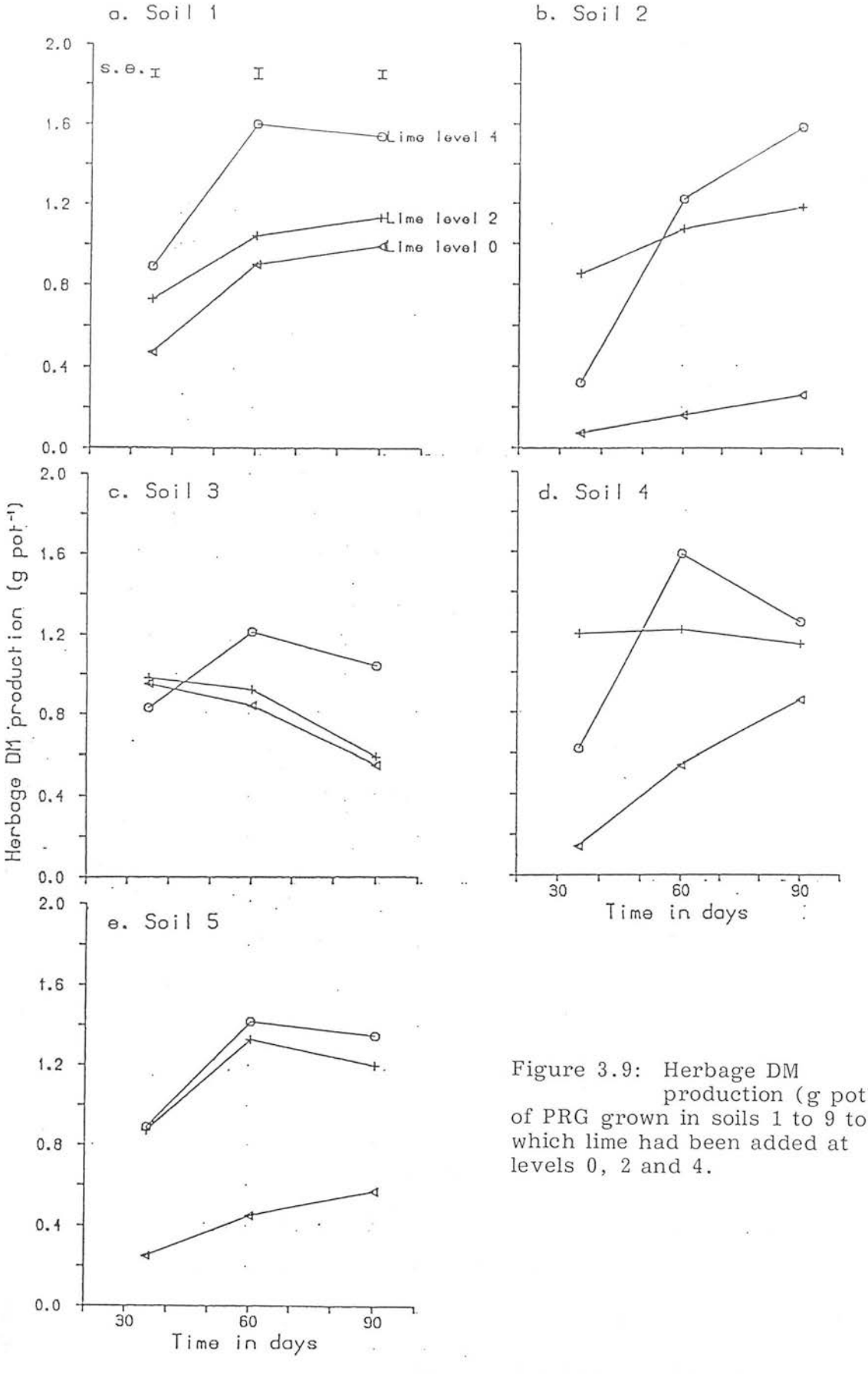

Figure 3.9: Herbage DM production (g pot^{-1}) of PRG grown in soils 1 to 9 to which lime had been added at levels 0, 2 and 4.

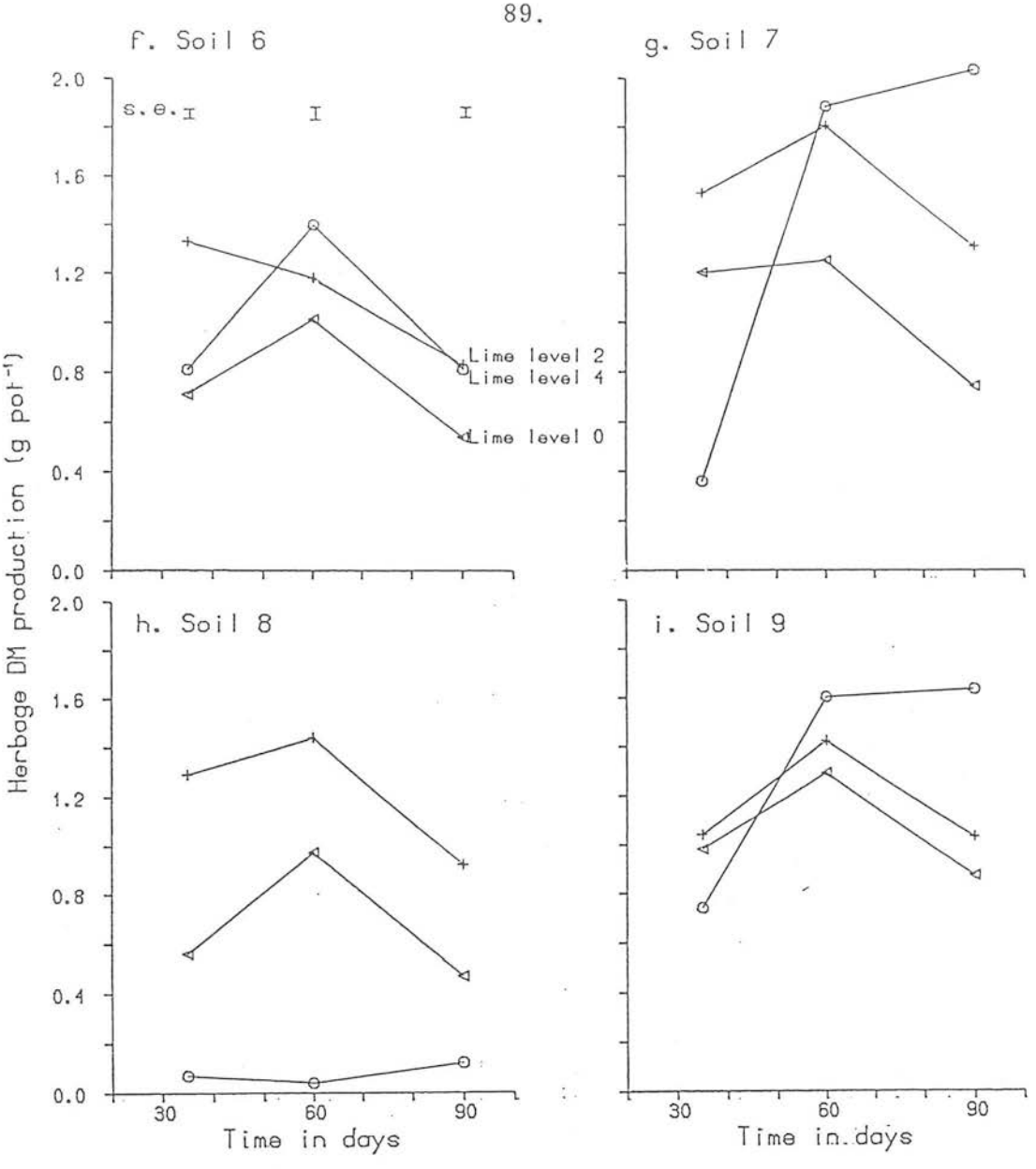

Figure 3.9 (continued)

which showed a decrease). But at the third harvest this trend was reversed for soils 6, 8 and 9, leading to decreased overall production (the first harvest compared to the last harvest). Decreased overall production was also found in the unlimed soils 3 and 7, also due to a drop in production between the second and third harvests. The other unlimed soils (1, 2, 4 and 5) all exhibited increased overall production, with increased production between the second and third harvests apart from soil 1.

The changes in DM production with time at lime level 2, were less consistent than those at the other two lime levels. Most soils showed increased production between the first and second harvest, although soils 3 and 4 showed no significant change and soil 6 a decrease. Between the second and third harvests all soils had decreased production except for soil 2 - an increase and soils 1 and 4 - no change. Thus, overall, four soils (numbers 3, 6, 7 and 8) had decreased production, three (numbers 1, 2 and 5) increased production and two (numbers 4 and 9) no change in production.

Dry matter production - Part B

The production from the three soils followed the same pattern with regard to the effect of lime addition and changes with time (Figure 3.10 and Table A3.10) as had been found in Part A. As the amount of lime present increased, unlimed to lime level 3 - lime level 4 was overlimed, so production increased at each harvest. The production at the additional lime levels 1 and 3 lay respectively below and above that at lime level 2. For soils 2 and 4 production at lime level 4 rose above that of the other lime levels by the final harvest, but the plants in soil 8 at lime level 4 suffered from overliming throughout the experiment.

The pattern of change in production with time varied in the three soils (see Figure 3.10).

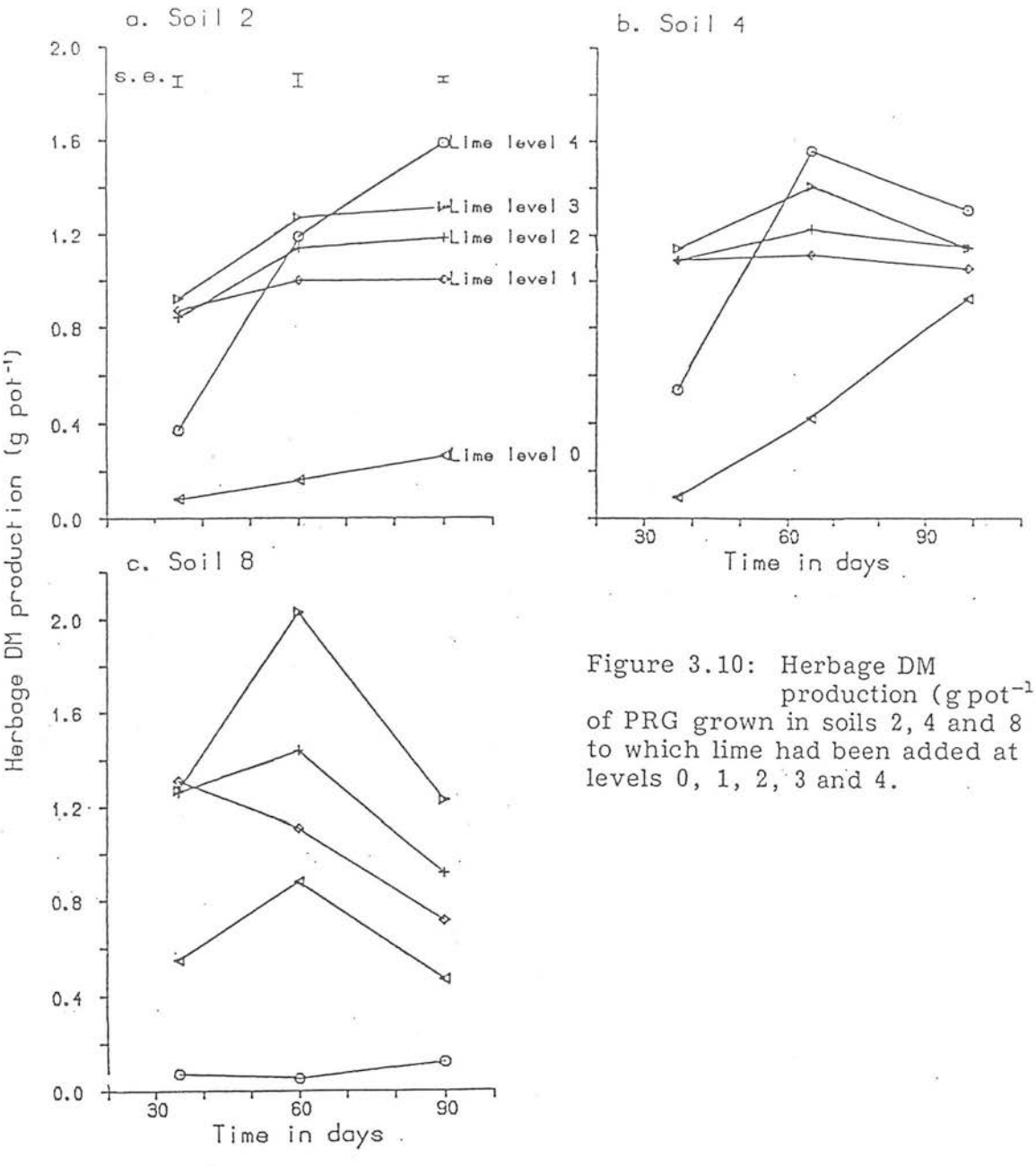

Figure 3.10: Herbage DM production ($g\ pot^{-1}$) of PRG grown in soils 2, 4 and 8 to which lime had been added at levels 0, 1, 2, 3 and 4.

Copper - Part A

There were large soil differences in herbage Cu concentration which were particularly pronounced at the first harvest (Figure 3.11 and Table A3.11). PRG grown in soil 8 contained the least Cu at all lime levels for all three harvests (range 0.5 - 3.4 mg Cu kg^{-1} DM) and that grown in soils 1 or 2 the most. PRG grown in soil 1 had the highest Cu content for lime levels 0 and 2 at the first and second harvest (range

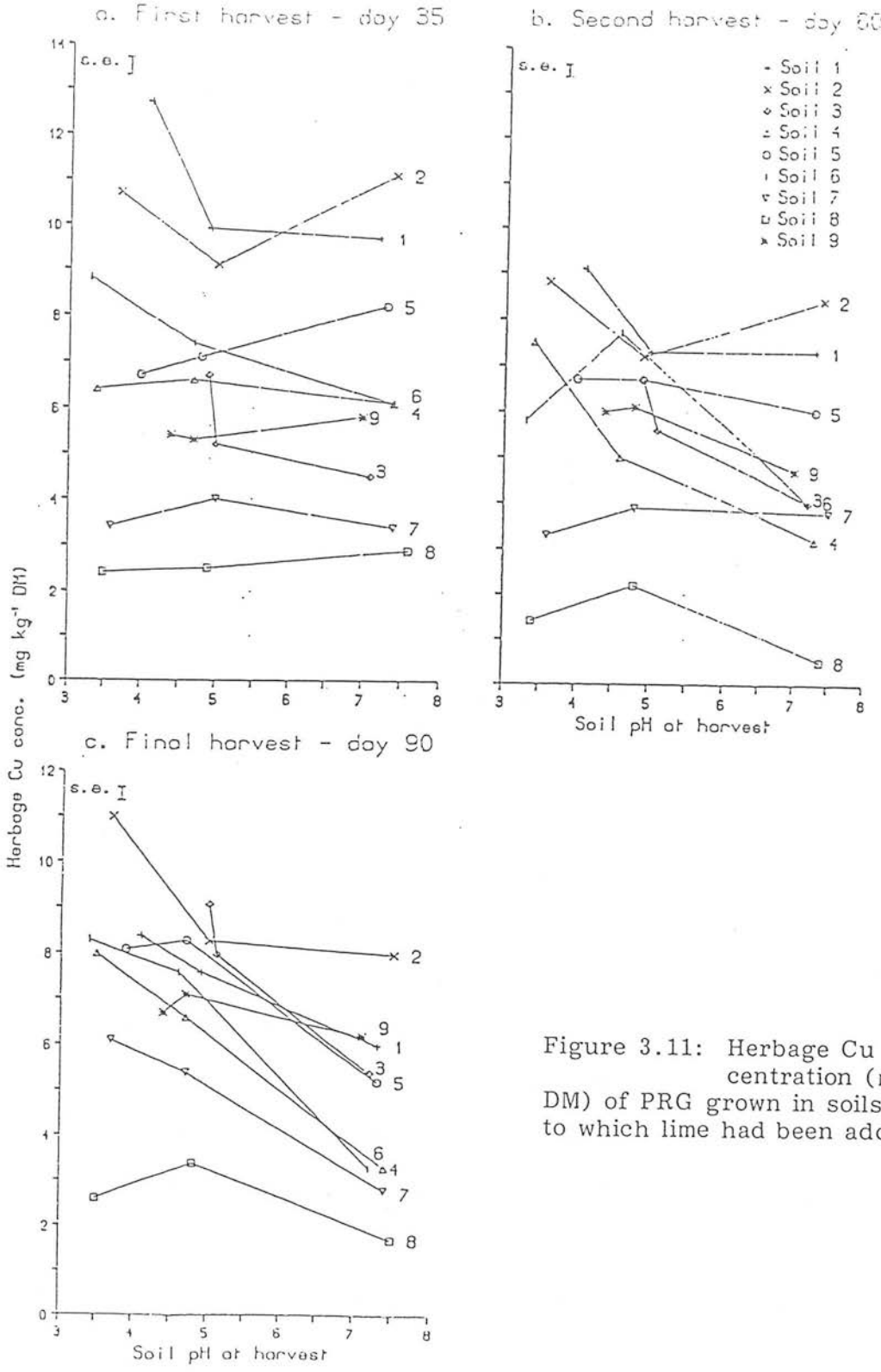

Figure 3.11: Herbage Cu concentration (mg kg^{-1} DM) of PRG grown in soils 1 to 9 to which lime had been added.

7.3 - 12.7 mg Cu kg^{-1} DM) and that grown in soil 2 the highest for lime level 4 at all three harvest and lime levels 0 and 2 at harvest 3 (range 8.0 - 11.1 mg Cu kg^{-1} DM). The range of herbage Cu concentrations considering all soils at all lime levels at the three harvests varied 26-fold.

The effect of lime on herbage Cu concentration varied over the course of the experiment. At the first harvest the effect of lime application was small and inconsistent (see Figure 3.11a). At the second harvest herbage growing in all soils exhibited decreased overall Cu content, except for herbage in soils 2 and 7 for which there was no difference in Cu content between lime levels 0 and 4 (for soil 2 this masked a decrease followed by an increase) - the pattern the overall decrease took varied with soil (see Figure 3.11b).

At the final harvest (Figure 3.11c), except for soil 9 where no overall change was observed, lime application consistently decreased herbage Cu content and the size of the decrease was greater than at the previous harvests. For example, for soil 4 herbage Cu contents were 8.0, 6.6 and 3.3 mg kg^{-1} DM at lime levels 0, 2 and 4 respectively at the third harvest compared to 7.5, 5.0 and 3.2 at the second harvest.

The range of herbage Cu concentrations observed tended to narrow during the course of the experiment with most values lying in the range 2 - 11, 3 - 8 and 3 - 9 mg Cu kg^{-1} DM at the first, second and third harvests respectively. For the different lime levels the Cu concentration of PRG growing in most soils tended to show no significant change or an increase during the course of the experiment in both the unlimed soil and at lime level 2, and to decrease at lime level 4.

Copper - Part B

The Cu contents of PRG growing in each of the three 'special' soils were in separate ranges and, as for extractable soil Cu, were in the order soil 2 > soil 4 > soil 8 (Table A3.12).

For soil 2 lime addition had no overall effect (L0 *cf.* L4) on herbage Cu content at the first harvest, although there was a significant increase between levels 2 and 3 after an apparent initial decrease. Likewise at the second harvest there was no overall effect of lime addition on the Cu concentration in PRG but there was a decrease (from level 0 to 1) followed by an increase (from levels 2 to 3, and 3 to 4) back to the initial concentration. At the final harvest there was an overall decrease in PRG Cu content brought about by decreases between levels 0 and 1, 1 and 2 (Figure 3.12).

For herbage growing in soil 4 there was no significant overall difference in Cu concentration with lime additions at the first harvest since the apparent tendency for content to increase at low lime levels (non-significant between adjacent lime levels) was balanced by a decrease between levels 3 and 4. At the second harvest herbage Cu concentration decreased overall, as it also did at the third harvest - to a greater extent than in the previous harvest with significant decreases occurring between all lime levels (Figure 3.12).

The Cu concentration of herbage growing in soil 8 was unaffected by lime application at the first harvest and at the second harvest there was no net effect, although some minor fluctuations occurred (Figure 3.12). At the final harvest there was an overall decrease in herbage Cu concentration, the initial increase (L0 to L1) being followed by a steady decrease between all the other lime levels (Figure 3.12).

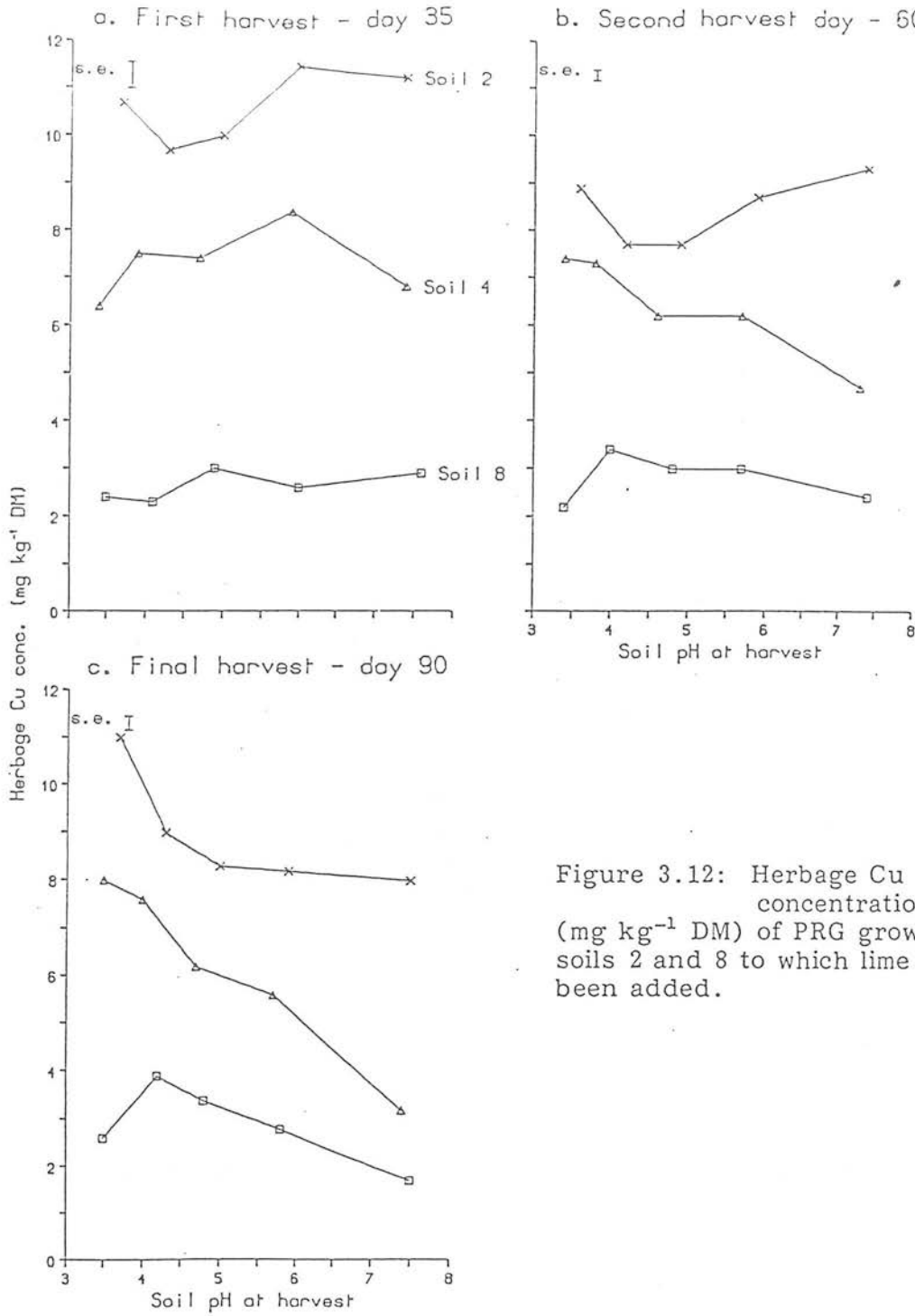

Figure 3.12: Herbage Cu concentration (mg kg^{-1} DM) of PRG grown in soils 2 and 8 to which lime had been added.

The changes in herbage concentration with time were similar to those exhibited in Part A - a narrowing of the range of values found and more pronounced decreases, especially at high lime levels, towards the end of the experiment.

For herbage growing in soil 2 there was no overall (first harvest compared to third) change in Cu content at lime levels 0 and 1, comprising a decrease between the first and second harvests and an increase between the second and third. At the other three lime levels herbage Cu concentrations decreased overall - a straightforward decrease at level 4, a decrease and then no change at level 3 and a decrease followed by a small increase at level 2.

The Cu concentration of herbage growing in soil 4 exhibited an overall progressive increase at lime level 0, no change at lime level 1, a decrease at lime level 2 (a decrease followed by no change) and overall progressive decreases at lime levels 3 and 4. Therefore, although for herbage growing in the three soils considered as a set the range of herbage Cu values narrowed with time for herbage growing in soil 4 the range widened.

The Cu concentrations of herbage growing in soil 8 besides being the lowest in the Experiment (Parts A and B) with most lying in the range 2 - 3 mg Cu kg^{-1} DM, also showed the least change during the experiment. There were no significant changes in the herbage concentrations for lime levels 0, 2 and 3, whilst for level 1 there was an overall progressive increase and for level 4 an overall decrease which occurred in the final growth period between the second and third harvests.

Molybdenum - Part A

The addition of lime to the soils generally led to a large increase in herbage Mo concentration and the size of this increase itself increased during the course of the experiment. At the first harvest the range of Mo contents for herbage growing in the unlimed soils was 0.18 to 1.3 mg Mo kg^{-1} DM compared to 0.71 to 4.0 mg Mo kg^{-1} DM at lime level 4

and for the last harvest the ranges were 0.92 - 2.8* and 2.0 - 8.0 mg Mo kg^{-1} DM respectively (Table A3.13).

At the first harvest the addition of lime led to increased overall (L0 *cf.* L4) herbage Mo content in all soils. For soils 1, 3, 4 and 9 the increase occurred only between lime levels 2 and 4 and for soil 8 only between levels 0 and 2 but for the remaining four soils (2, 5, 6 and 7) there was an increase with both additions of lime (Figure 3.13a).

At the second harvest herbage growing on all soils except soils 4 and 8 still exhibited an overall increase in Mo concentrations (Figure 3.13b). For soils 4 and 8 there was no significant overall change in content, but both soils showed increased Mo content with the first lime addition and then a decrease with the second lime addition (the decrease was non-significant for soil 4).

By the third harvest only herbage growing in soils 1, 2, 5, 6, 8 and 9 still had higher Mo contents after liming (L0 *cf.* L4) (Figure 3.13c). Herbage growing in soil 3 showed a large decrease which occurred between lime levels 0 and 2 and herbage growing in soils 4 and 7 had no significant overall change in Mo content despite small increases in soil 4 (L0 to L2). For the six soils in which herbage had increased Mo contents only soil 2 produced significant increases with both lime additions, soil 6 and 8 produced significant increases between lime levels 0 and 2 and soils 1, 5 and 9 between levels 2 and 4.

Although there was a tendency for some unlimed soils (3, 4, 7 and 9) to produce herbage with significantly increased Mo content over the course of the experiment the effect was a lot larger where lime had been applied. At lime level 2 all soils, except for soil 5, produced

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*Excluding herbage growing in soil 3 which contained 8.5 mg Mo kg^{-1} DM.

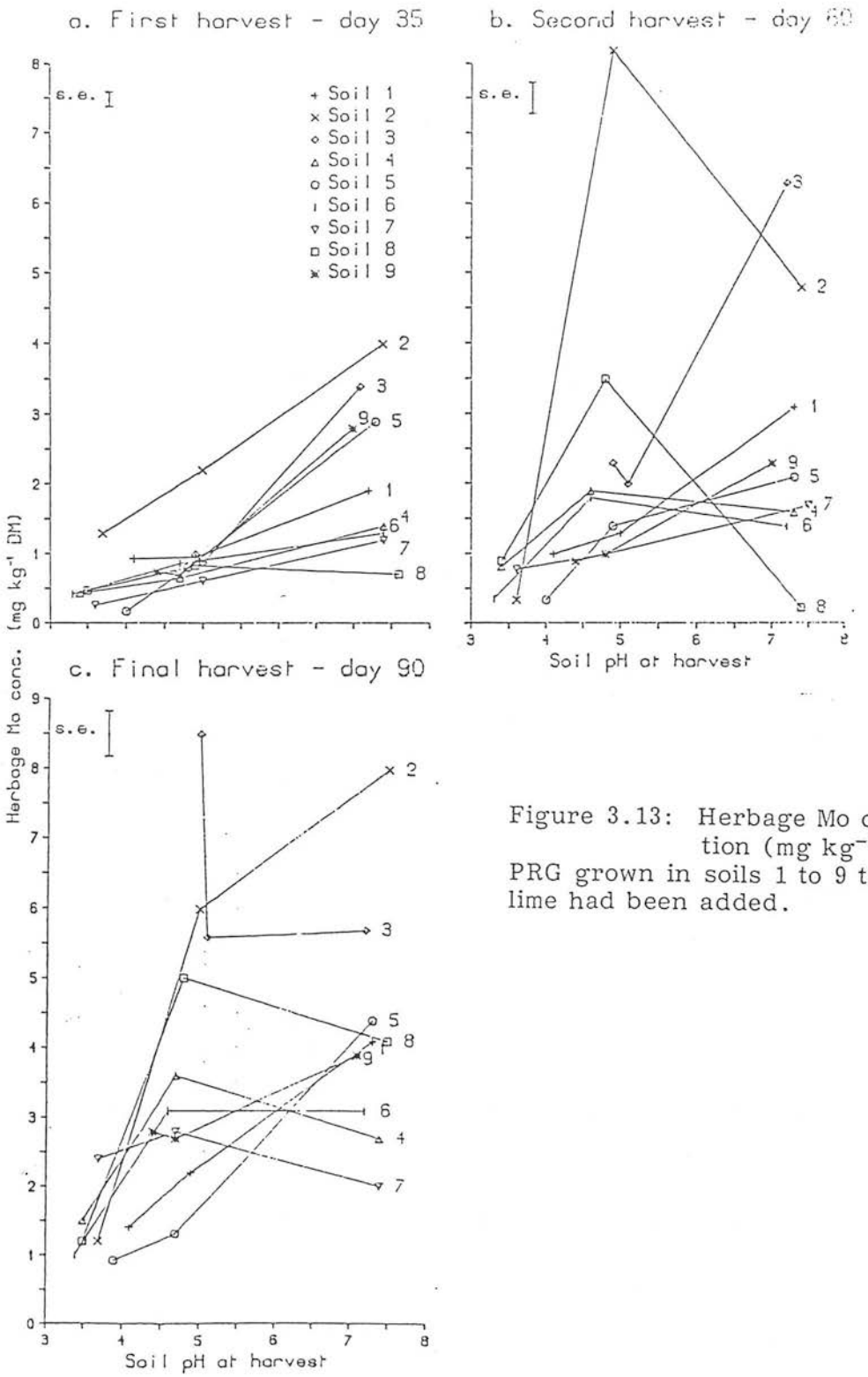

Figure 3.13: Herbage Mo concentration (mg kg^{-1} DM) of PRG grown in soils 1 to 9 to which lime had been added.

herbage which had increased Mo content over the course of the experiment. The size of the increase in herbage Mo content produced at lime level 4 was larger than that at level 2 except for herbage grown in soil 7 which showed no significant change.

Molybdenum - Part B

The same trends of increased herbage Mo concentration with (a) lime application to soil and (b) the course of the experiment that had been noted in Part A were also seen in Part B. The effects were far larger in soil 2 than in soils 4 and 8 (Figure 3.14 and Table A3.14).

At the first harvest (Figure 3.14a) herbage grown in soil 2 had increased overall Mo content; increases occurred between lime levels 1 and 2 (approximately 70% increase) and 2 and 3 (approximately 300% increase) and a decrease (approximately 35%) from level 3 to 4 - the overlimed treatment. In soil 4 although the increases between lime levels were not themselves significant the overall increase (four-fold) was; also, although the herbage produced on soil 4 contained less Mo than that produced on soil 2 the relative percentage increase between lime levels 0 to 4 was of the same order - 400%. Herbage grown in soil 8 showed no significant overall change in Mo content despite small non-significant increases from lime levels 0 to 1, 1 to 2 and 2 to 3 and a significant decrease from level 3 to 4.

At the second harvest (Figure 3.14b) herbage grown on soil 2 showed an overall sixteen and a half-fold increase in Mo content with significant increases occurring between all lime levels except 3 and 4. Herbage grown on soil 4 showed no overall increase in Mo content as was also the case for herbage grown on soil 8 although this masked an increase in lime level 0 to 1 and a decrease in level 3 to 4.

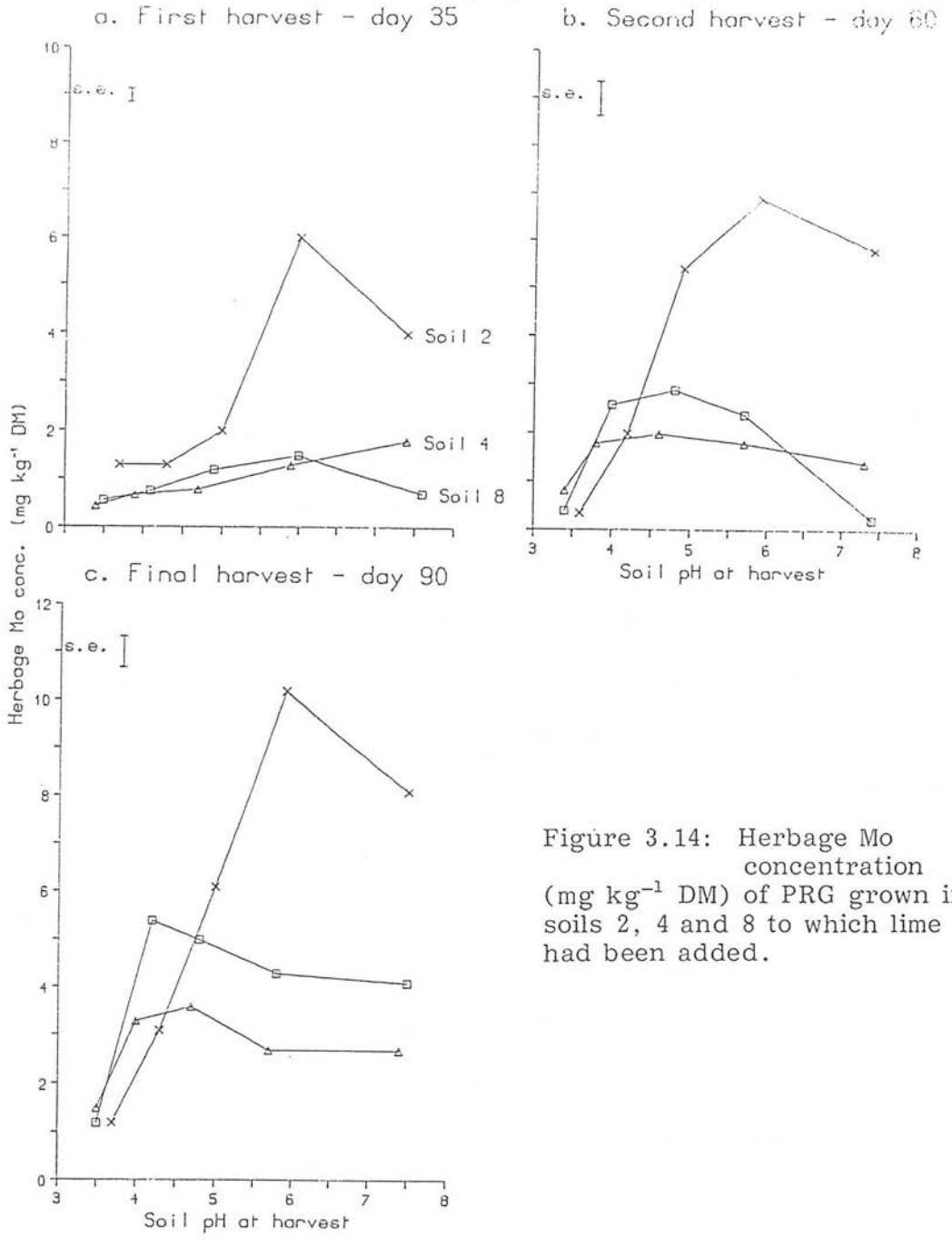

Figure 3.14: Herbage Mo concentration (mg kg^{-1} DM) of PRG grown in soils 2, 4 and 8 to which lime had been added.

At the third harvest (Figure 3.14c) the concentration of Mo in herbage grown on soil 2 increased greatly between lime levels 0 to 1, 1 to 2, and 2 to 3 and decreased between levels 3 and 4, the overall increase was six and three-quarter-fold and that from level 0 to 3 eight and a half-fold. For herbage grown on soils 4 and 8 there were increases in Mo content between levels 0 and 1 (non-significant for soil 4) followed by no further significant changes.

There were significant overall increases in the Mo content of herbage grown in the three soils between the first and third harvests for all lime applications (except for soil 4 L4), and also for the unlimed soil 4, although the increases between the harvests were individually non-significant (Figure 3.4).

Sulphur - Part A

Lime addition to a soil was generally found to increase the concentration of S in PRG growing in the soil (Table A3.15). At the first harvest (Figure 3.15a) all soils, except soil 8, produced herbage with increased S contents on liming, either between lime levels 0 and 2 (soils 1, 2, 5 and 7), or levels 2 and 4 (3, 6 and 9)[1], or both (soil 4). Apart from herbage produced on soils 1 and 5 the overall increase (L0 to L4) was significant, in the case of soil 6 the large increase (approximately 100%, i.e. 0.24-0.45%) between lime levels 2 and 4 outweighed the smaller decrease (0.32 - 0.24%) from lime level 0 to 2.

At the second harvest (Figure 3.15b) the range of S contents found in the herbage had decreased to 0.11 - 0.33%[2] from 0.22 - 0.41%[3] at the first harvest, and lime addition was still found to generally cause an overall increase in herbage S%. Increased herbage S concentrations between lime levels 0 and 2 were found on soils 2 and 7 although decreases also occurred with this lime addition (soils 4, 5 and 8) but no significant change was the most common effect (soils 1, 3[1], 6 and 9). Herbage growing in all soils, except soils 2 and 5, had increased S percentages between lime levels 2 and 4 - soils 2 and 5 had decreased percentages.

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[1] NB for soils 3 and 9 the pH at lime levels 0 and 2 was very close.

[2] Excluding 0.39% for soil 8 at lime level 4.

[3] Excluding two high values of 0.45% and 0.54% found in soils 6 and 4 respectively at lime level 4.

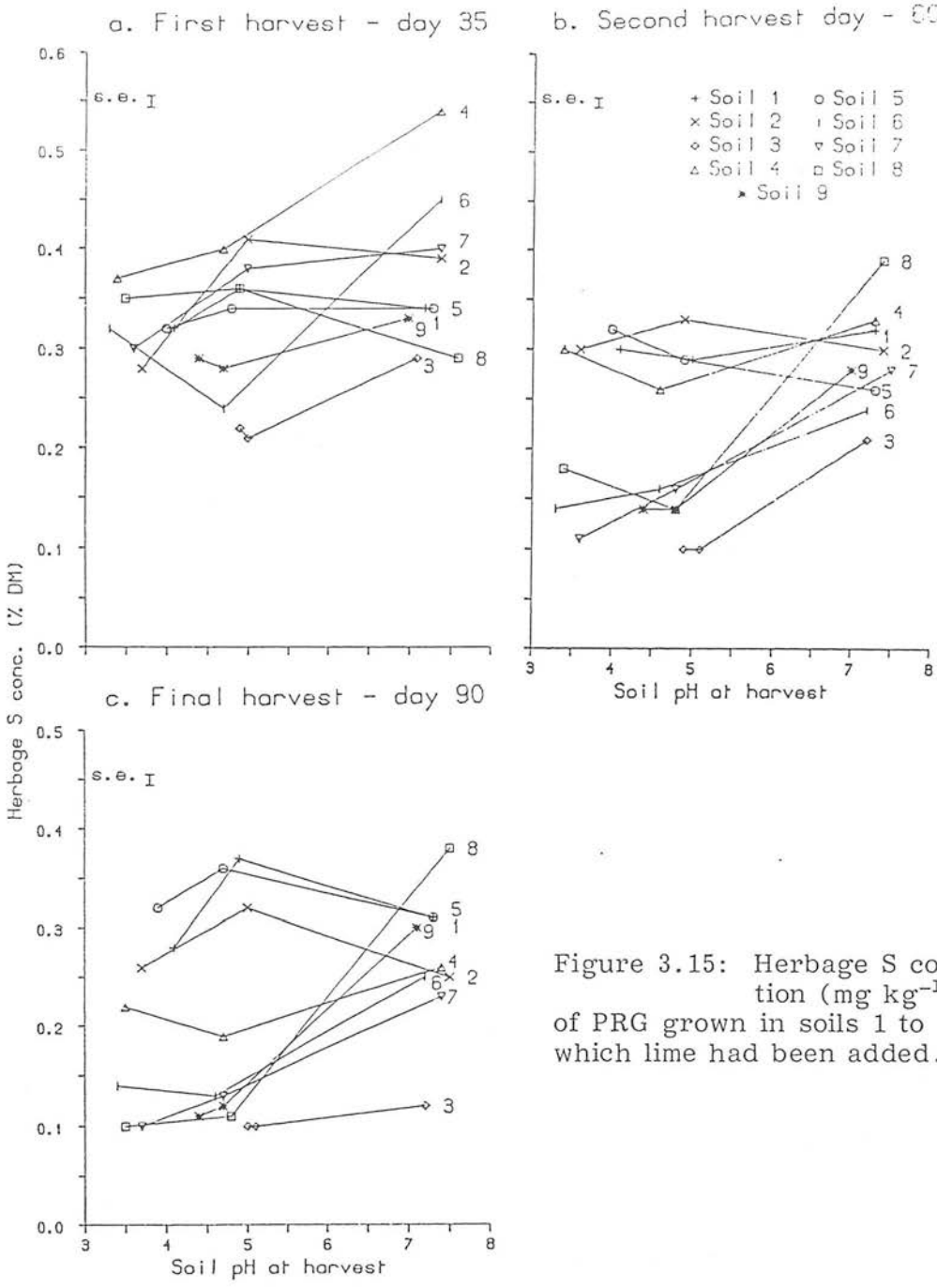

Figure 3.15: Herbage S concentration (mg kg^{-1} DM) of PRG grown in soils 1 to 9 to which lime had been added.

At the third harvest (Figure 3.15c) most soils (1, 4, 6, 7, 8 and 9) still produced herbage whose S% increased overall with lime addition although soils 2, 3 and 5 produced no overall significant effect on herbage S% - for soils 2 and 5 an increase between lime levels 0 and 2 was balanced by a decrease between levels 2 and 4, whilst herbage produced on soil 3 was unaffected by either lime addition.

As already noted above there was a tendency for herbage S% to decrease during the experiment and only one treatment (soil 8, lime level 4) did the overall S% increase between the first and third harvest, in all other cases the % decreased (the majority of treatments) or remained unchanged (soils 2 and 5 at lime level 0 and soils 2 and 5 at lime level 2). For most treatments the decrease occurred mainly between the first and second harvests and there was a far smaller less frequent decrease between the second and third harvests.

Sulphur - Part B

The more detailed study of soils 2, 4 and 8 revealed no overall differences to the trends noted in Part A of increased herbage S% with lime addition and decreased contents between the first and second harvest (Table A3.16).

At the first harvest herbage grown on soils 2 and 4 had overall increased S concentrations (L0 to L4) (Figure 3.16a). For herbage grown on soil 2 all the increase occurred between levels 1 and 2, and 2 and 3 whilst for soil 4 a small initial decrease (L0 to L1) was followed by large increases between levels 2 and 3 and 3 and 4. The overall increase in both soils was about 50%. Soil 8 produced herbage with an overall decrease in S%, the decrease occurred between lime levels 1 and 0, and 3 and 4 (the overlimed treatment) although there were increases between levels 1 and 2, and 2 and 3 such that the S% at lime level 3 was higher than that in the unlimed control.

At the second harvest (Figure 3.16b) the S contents were lower than at the first harvest and on soil 8, alone, produced herbage whose overall (L0 *cf.* L4) content increased with lime addition. The herbage grown in soil 2 had an overall decrease in S% arising from decreases

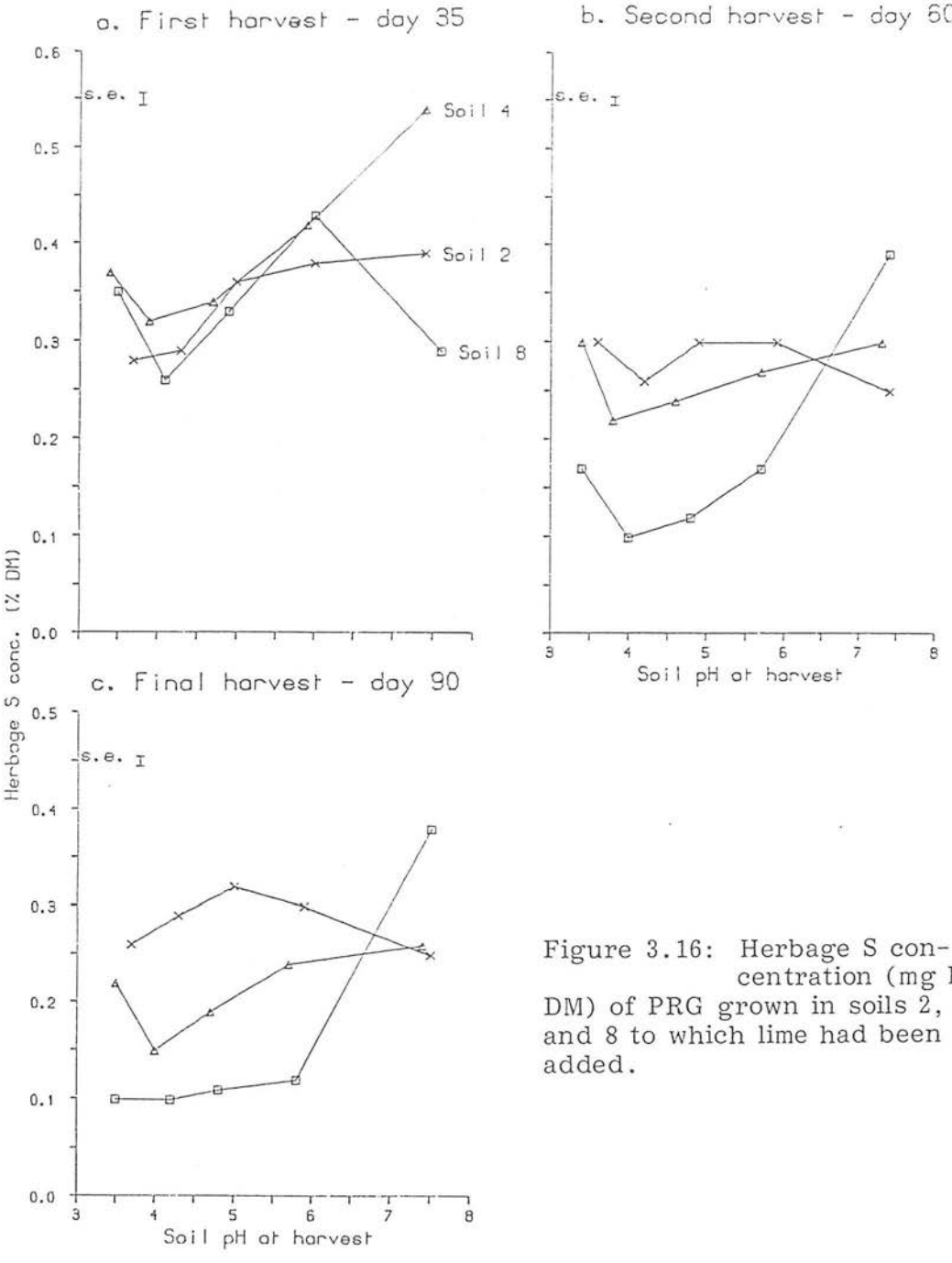

Figure 3.16: Herbage S concentration (mg kg^{-1} DM) of PRG grown in soils 2, 4 and 8 to which lime had been added.

between lime levels 0 and 1, and 3 and 4, which the increase between levels 1 and 2 was not great enough to overcome. Soil 4 produced herbage with no significant overall change in S%. For herbage grown on soil 8 the decrease in S% between lime levels 0 and 1 was smaller than the subsequent increases between levels 1 and 2, 2 and 3 and

3 and 4, the last increase was especially large. Overall (level 0 to 4) there was a 130% increase in soil 8, and considering the increase between levels 1 and 4 the increase was 300%.

At the third harvest (Figure 3.16c) herbage grown on soil 2 showed no overal significant change in S%, as did that grown on soil 8, although there were no significant changes between lime levels 0 and 1, 1 and 2 and 2 and 3 there was a large two-fold increase between levels 3 and 4 for this soil.

The S% in herbage from each treatment generally decreased throughout the experiment, but soil 8 at lime level 4 produced an increase (as in Part A) and soil 2 at lime levels 0 and 1 no change (Table A3.16).

2.2 Experiment 2: *An investigation into the effect of lime application on the content of copper, molybdenum and sulphur in perennial ryegrass and white clover grown in soils 2, 4 and 8; with two methods of lime application for perennial ryegrass.*

The basic experimental design was:

2A 3 soils (2, 4, 8) x 3 pH (lime levels 1, 2, 3 - L1, L2, L3) x 2 species (WC, PRG) x 4 replicates with 2 sequential harvests on days 40 and 70.

2B 3 soils (2, 4, 8) x 3 pH (lime levels 1, 2, 3 - L1, L2, L3) x 2 methods of lime application (Lime A and Lime S) x 4 replicates growing PRG with 2 sequential harvests on days 40 and 70 (Full details are given in Chapter II, Section 4.1).

The experiment was originally designed to investigate the effect of method of lime application in both species, however the DM production of WC in Lime S pots was very poor, no growth occurred at lime level 3. Hence there was not generally sufficient herbage for chemical analysis and, for statistical analysis the WC Lime S pots were discarded and the experiment split into two halves - A and B - as above.

The data for the experiment is mainly presented as figures; pH is plotted against time, DM production is presented as histograms and all other variables are plotted against the soil pH at the time the herbage sample was taken. The measured soil pH was found to decrease during the experiment, particularly for PRG Lime S pots (see pH section for further details), and this should be borne in mind whilst studying the figures, and also the tables of data presented in Appendix 3.2.

The lower production at the first harvest for PRG at Lime S level 3 in soils 2 and 8 and for all WC treatments, particularly lime level 3, must be borne in mind when assessing nutrient uptake. For Part

B only the results at the first harvest are presented for Cu, S and Ca because of the 'inaccurate' pHs at the second harvest and also because the plants were beginning to appear slightly unhealthy towards the end of the experiment. The results at both harvests are presented for Mo although the above considerations also apply because the two harvests gave very different results.

(a) Soil analysis

Soil pH - Part A

The addition of the calculated amounts of lime to the three soils was successful in giving three distinct initial soil pHs which, unlike Experiment 1, were very close to the 'desired' pHs - 4.5, 5.5 and 6.5. There was a soil effect and a soil x lime interaction on pH at all three sampling times with soil 8 having lower pHs than soils 2 and 4 (Figure 3.17 and Table A3.17).

The plant species growing in the soil also had an effect on the soil pH at the start and end of the experiment - soils growing grass having higher pHs at both times by 0.1 or 0.2 units. The difference at the start of the experiment was probably due to different techniques of sampling being used for the two species (see Chapter II, Section 4.2), and the difference at the end occurred because legumes have an ability to acidify the medium in which they are growing (Haynes, 1983).

The soil pH of all treatments decreased over the course of the experiment. The magnitude of the decrease was unaffected by the soil but was larger the higher the initial soil pH, with the pH at lime level 1 decreasing by only 0.2 units and that at level 3 by 0.4 - 0.6 units. For the pots in which PRG was grown the decrease occurred

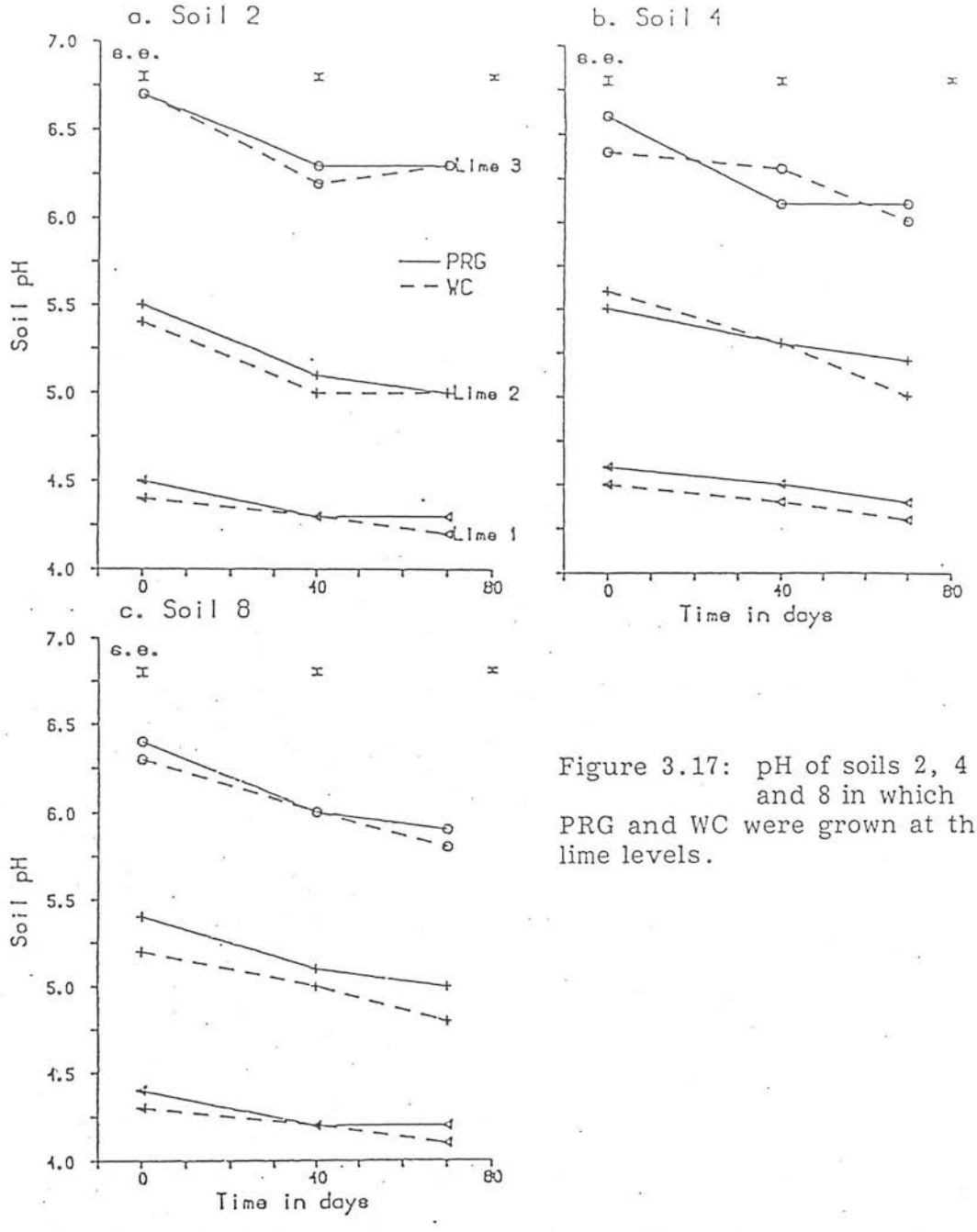

Figure 3.17: pH of soils 2, 4 and 8 in which PRG and WC were grown at three lime levels.

almost completely between the start of the experiment and the first harvest, whilst for WC it continued up to the second harvest, reflecting this species' ability to acidify the soil.

The reduction of soil pH during the course of a pot experiment is frequently observed and is due to: increased concentrations of H^+ ions in the soil brought about by the nitrification of NH_4^+ to NO_3^- (the N source was NH_4^+ NO_3^-) and by the plant excretion of protons associated with cation (including NH_4^+) uptake exceeding the excretion of HCO_3^- associated with anion uptake, i.e. altered cation/anion balances (Helyar, 1976). That pH did not decrease in a similar fashion during Experiment 1 could be because whereas Experiment 2 was carried out during the spring Experiment 1 was carried out during the winter when, although in a glasshouse, the soil would have been colder and rates of nitrification less. This supposition is supported by the dry weight data (see Chapter IV, Section 6), and also by the evidence of Floate and Pimplaskar (1976) that mineralization of 'P' from hill soils was less in winter than in summer glasshouse pot experiments.

Soil pH - Part B

The soil samples on which the pH analyses were performed were taken by a different technique at each of the three sampling times (see Chapter II, Section 4.2) and it is necessary to remember this when studying the pH in the surface applied lime treatments.

The pH of the initial sample was greater in the pots where lime had been applied to only the top 30% (by volume) (Lime S) than in the pots where lime was applied throughout the soil (Lime A). The difference was on average 1.6 units at lime level 1, 1.8 at level 2 and 1.2 at level 3, the smaller increase at lime level 3 was due to the plateau of the lime response curve having been reached (Figure 3.18, Table A3.17).

At the first harvest the pH of the Lime S pots was still greater than the Lime A pots but the size of the difference was smaller; on

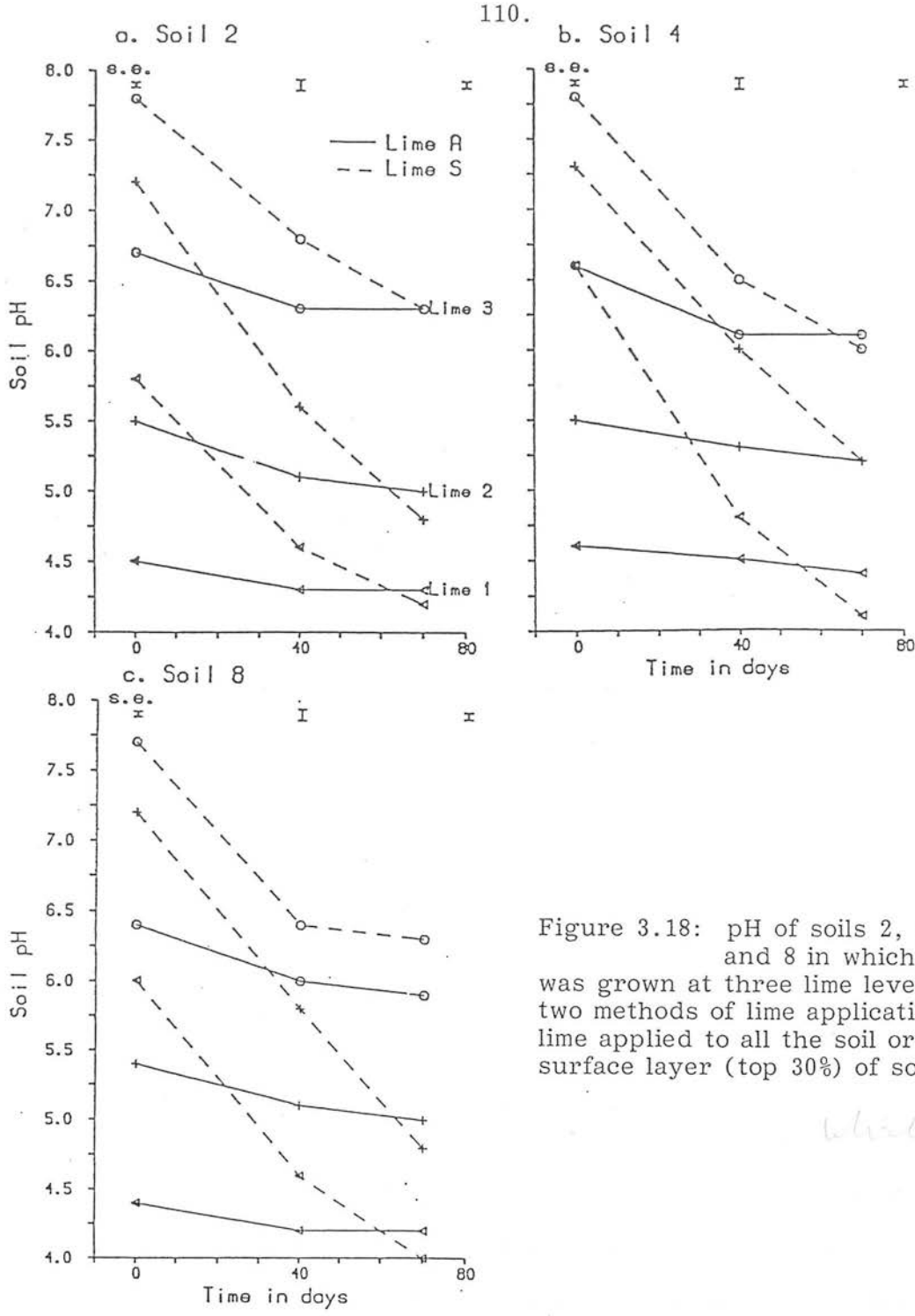

Figure 3.18: pH of soils 2, 4 and 8 in which PRG was grown at three lime levels with two methods of lime application - lime applied to all the soil or to the surface layer (top 30%) of soil.

average 0.3 units at lime level 1, 0.7 at level 2 and 0.5 at level 3. Although the samples at the start of the experiment were taken in a different manner to those at the first harvest both were assessing the region of the soil to which lime had been added in the Lime S pots, so differences in sampling technique do not explain the reduction in

soil pH. Instead the reduction could be due to the lime having been slowly transferred down the pot because of the surface watering and plant growth, combined with the possible production of plant root exudates, soil nitrification activities and altered soil cation/anion balances which also led to the decrease of pH in the Lime A treatments (see above).

At the second harvest the method of lime application still had an effect but now all comparable Lime S and Lime A treatments were at the same pHs, or Lime S pots had pHs 0.1 to 0.2 units lower (except for soil 8 at lime level 3 where treatment Lime S was 0.4 pH units higher). The reason for the convergence of the pHs in the two lime application treatments is probably due entirely to differences in the sampling technique used *cf.* those for the previous two pH samples.

(b) Herbage analysis

Dry matter production - Part A

The WC plants took longer to become established than the PRG plants and so at the first harvest DM production was much lower for WC than for PRG. By the second harvest production was greater for WC than for PRG, except soil 2 at lime level 3 where PRG > WC and soil 2 at lime level 1 where PRG ≈ WC. The size of differences at both harvests varied with soil and lime level but was consistently much smaller at the second harvest - 0.2 to 0.9 g difference, compared to 1.0 to 2.25 g, which were percentage differences of approximately 10-50% and 100 to 1500% (Figure 3.19 and Table A3.18).

For PRG DM production increased as the amount of lime applied was increased. For WC at the first harvest lime level 3 produced the least production in all three soils and lime level 2 the most (the difference between L1 and L2 was not significant in soil 4). By the second harvest

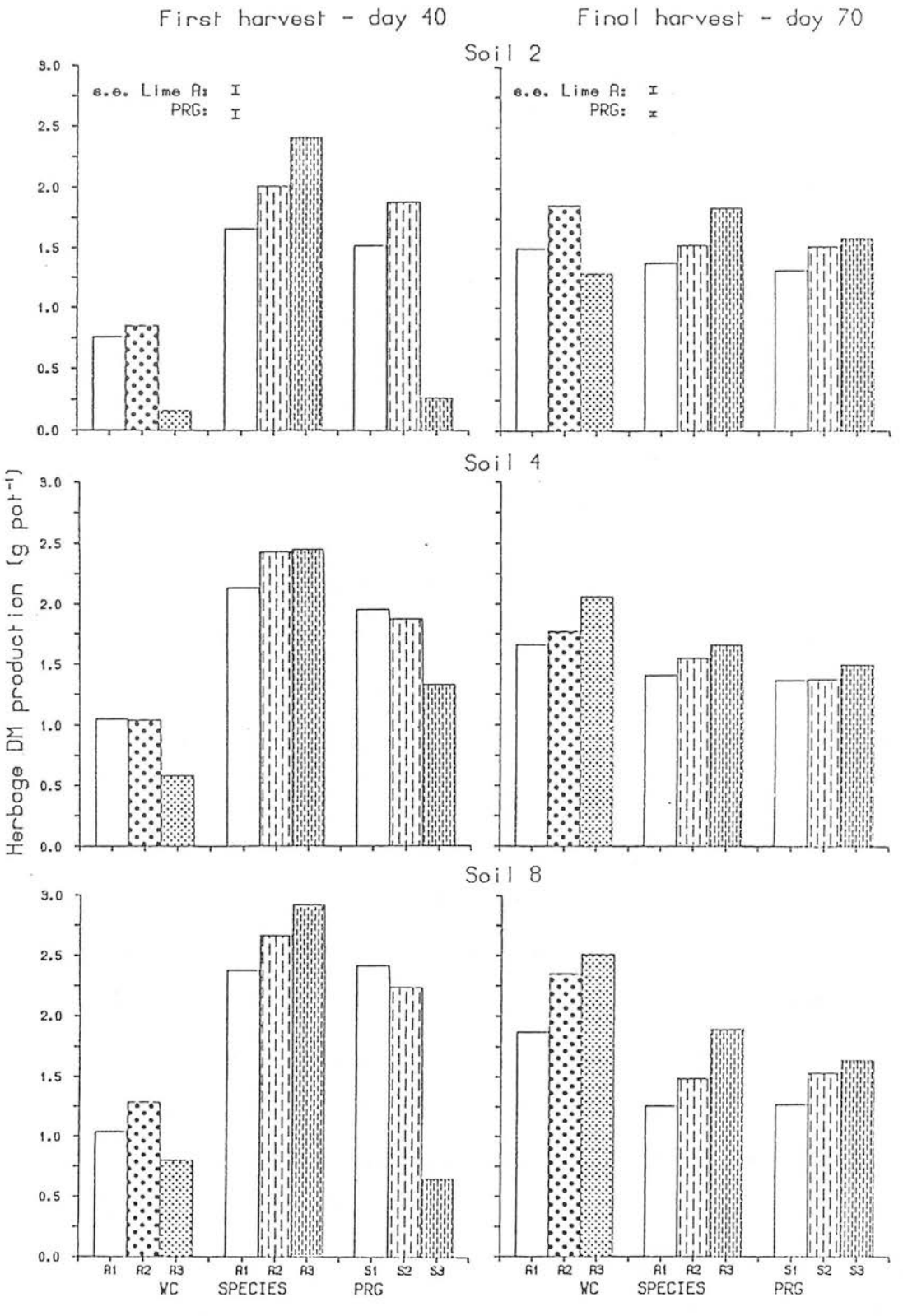

Figure 3.19: DM production (g pot^{-1}) of PRG and WC grown in soils 2, 4 and 8 at three lime levels with, for PRG, two methods of lime application - harvests taken after 40 and 70 days.

the adverse effects of the highest lime level on WC production had been overcome in soils 4 and 8 and the order of production with regard to the amount of lime that had been applied was the same as for PRG, i.e. level 3 > level 2 > level 1. In soil 2, however, lime level 3 was still producing an adverse effect on WC growth and the order of production with regard to lime level was as at the first harvest, i.e. level 2 > level 1 > level 3. Possible reasons for the highest lime level leading to decreased production in clover include salt effects which caused delayed establishment or to an induced P deficiency.

Dry matter production - Part B

The largest effect of lime application occurred at Lime S level 3 at the first harvest where production was greatly inhibited compared to all other treatments. This effect was less marked in soil 4 and by the second harvest was overcome in all soils (Figure 3.19 and Table A3.18). Some reasons for the inhibition of production have already been outlined above, and by the second harvest the adversely high pH was overcome not only because the pH of the surface layer declined (see page 110) but also because the plant roots were no longer mainly confined to the top heavily limed zone of soil. All treatments apart from Lime S at level 3 had reduced DM production at the second harvest, by which stage the plants were beginning to look unhealthy and the soil effect was no longer significant.

Pots to which lime had been applied by method A always produced more than, or had no significant difference in production from, Lime S pots. At lime level 1 there was no significant difference in production in any soil at either harvest, except soil 4 at the first harvest. At lime level 2 significantly greater production occurred in Lime A pots for

soil 4 at both harvests and soil 8 for the first harvest; whilst for lime level 3 all Lime A pots at both harvests had significantly greater production (see above). For Lime A pots production was always greatest at level 3 and least at level 1, as was also the case for Lime S at the second harvest.

Copper - Part A

Throughout the experiment the Cu concentration was higher in WC than in PRG for plants grown in soils 2 and 4, but not in soil 8 - the soil having the lowest initial Cu concentration. The magnitude of the difference was greater at the second harvest in both soils (by 1½ to 6 times), but particularly for plants growing in soil 4 at lime levels 1 and 2 and soil 2 at lime level 3 (Figure 3.20 and Table A3.19).

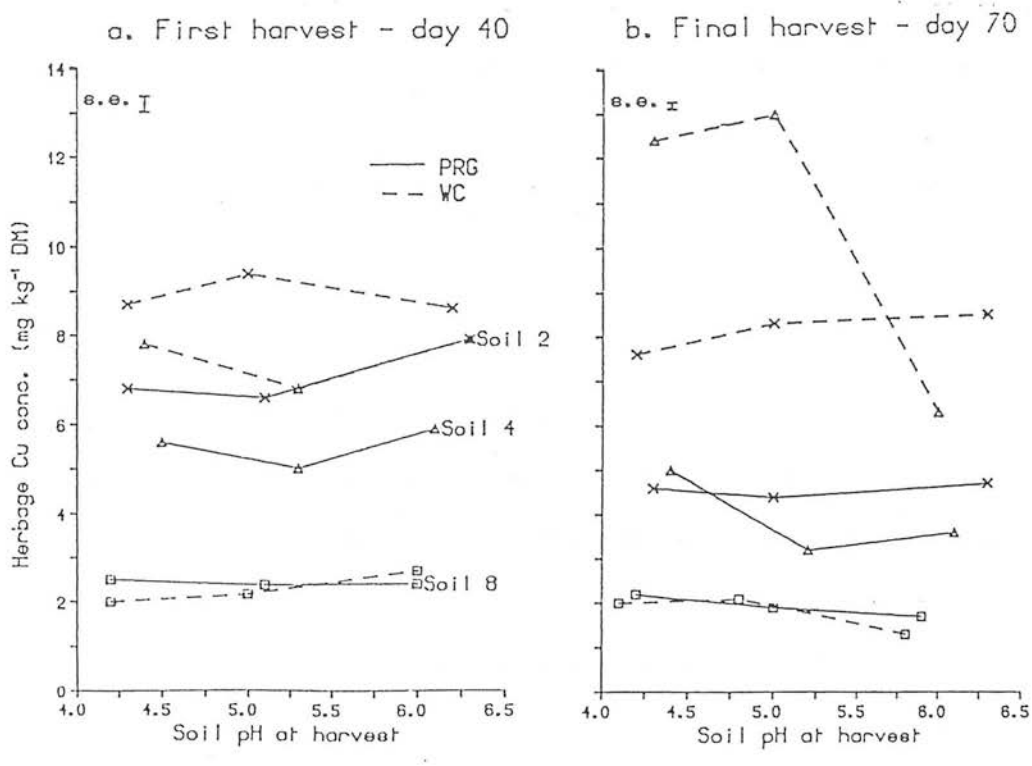

Figure 3.20: Herbage Cu concentration (mg kg^{-1} DM) of PRG and WC grown in soils 2, 4 and 8 to which lime had been added.

Lime addition had no significant effect on herbage Cu concentrations at the first harvest but had an effect at the second harvest, which was most noticeable in WC grown in soil 4. For WC growing in soil 2 the overall Cu content in the herbage (L1 compared to L3) increased, whilst PRG growing in the soil was not affected by lime application (in contrast to Experiment 1 where a decrease was noted but not until the third harvest). The Cu content of both PRG and WC grown on soil 4 decreased overall: the decrease was especially noticeable for WC where the concentration at lime level 3 (6.3 mg kg^{-1} DM) was approximately half that at level 1 (12.4 mg kg^{-1} DM); in PRG the decrease (5.0 to 3.2 mg kg^{-1} DM) occurred between lime levels 1 and 2 followed by a small, just significant, increase to level 3 (3.6 mg kg^{-1} DM) (in agreement with Experiment 1). For herbage grown on soil 8 there was no significant change of PRG Cu concentration with lime addition (no effect was also noted for the first and second harvests of Experiment 1), but there was a small significant decrease in WC content which occurred between lime levels 2 and 3.

Copper - Part B

Only the results for the first harvest will be considered here for the reasons given in the DM production section. The results for the second harvest are given in Table A3.19 and although generally lower than those for the first harvest (as was also found in Experiment 1) follow the same trends.

The same order of soils was observed as for Experiment 1 with the order of Cu concentration in PRG grown on the three soils, being soil 2 > soil 4 > soil 8 (Figure 3.21). Lime addition had no significant effect on the Cu content of herbage produced on soil 8 (Lime A and S)

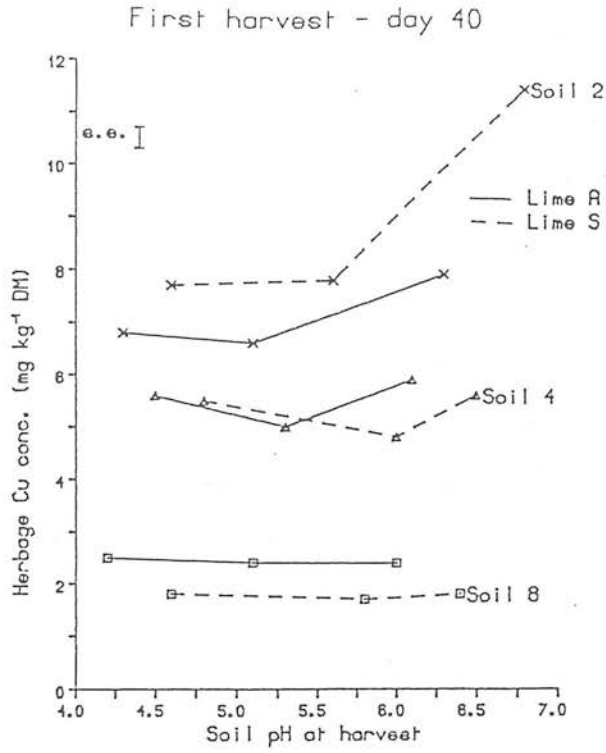

Figure 3.21: Herbage Cu concentration (mg kg^{-1} DM) of PRG grown for 40 days in soils 2, 4 and 8 to which lime had been applied either to all the soil or to the surface layer (top 30%) of soil.

but increased that of herbage produced on soil 2 (Lime A and S) and soil 4 (Lime A); in all three cases the increase occurred only between lime levels 2 and 3. An increase between lime levels 2 and 3 was also observed with Lime S in soil 4 but this was balanced by a decrease between the levels 1 and 2 leading to no significant overall change in herbage Cu concentration. The methods of lime application had no significant effect on herbage Cu concentrations.

Molybdenum - Part A

At the first harvest there was no significant difference at lime levels 1 and 2 between the Mo concentration of PRG or WC in any of

the three soils, but at lime level 3 WC contained more Mo than PRG (86% in soil 2, 76% in soil 4 and 96% in soil 8). At the second harvest only soil 2 at lime level 3 still produced WC containing more Mo than PRG, but the size of the difference was now half that at the first harvest (34%), and at lime level 2 in this soil WC contained less Mo than PRG. For the two species grown in soil 4 there was now no difference in Mo content at any lime level, and for herbage grown on soil 8 WC contained less Mo than PRG at all lime levels (although the difference was not significant at lime level 3) (Figure 3.22).

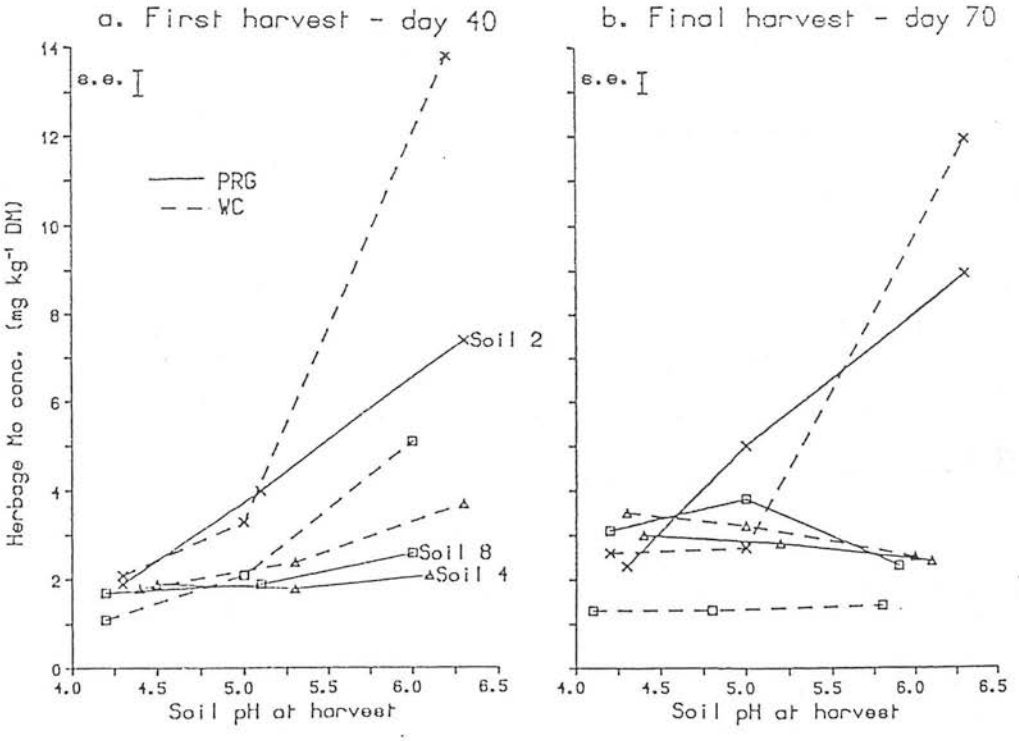

Figure 3.22: Herbage Mo concentration (mg kg^{-1} DM) of PRG and WC grown in soils 2, 4 and 8 to which lime had been added.

The effect of lime addition on herbage Mo concentration was much greater in soil 2 than in soils 4 and 8 as found in Experiment 1B, and were similar for PRG at the first harvest to those observed in Experiment 1B, i.e. herbage grown in soils 4 and 8 was unaffected by lime whilst that in soil 2 had increased Mo content. In all three soils the addition of lime elevated the Mo content of WC; for soils 4 and 8 at the first harvest the increase was only significant between levels 2 and 3 (Figure 3.22a). At the second harvest lime addition had no overall significant effect on the Mo content of either PRG or WC growing in soils 4 and 8, despite a small decrease for PRG in soil 8 between lime levels 2 and 3. For WC and PRG grown in soil 2 the application of lime greatly increased herbage Mo content, but for WC all the increase occurred between lime levels 2 and 3 (Figure 3.22b).

Broadly speaking, the range of values found for all three soils remained the same throughout the experiment with most treatments producing herbage with Mo contents in the range 1 - 4 mg kg^{-1} DM (Table A3.20).

Molybdenum - Part B

Data is presented for both harvests (Figure 3.23), as although the effect of method of lime application had no significant effect on herbage Mo concentration at the first harvest there was a very highly significant effect at the second with Lime A consistently increasing herbage Mo content in soils 4 and 8 compared to Lime S. The effect of lime application method was more difficult to interpret in soil 2, which produced herbage with the highest Mo concentrations as it was confounded with the effect of level of lime addition.

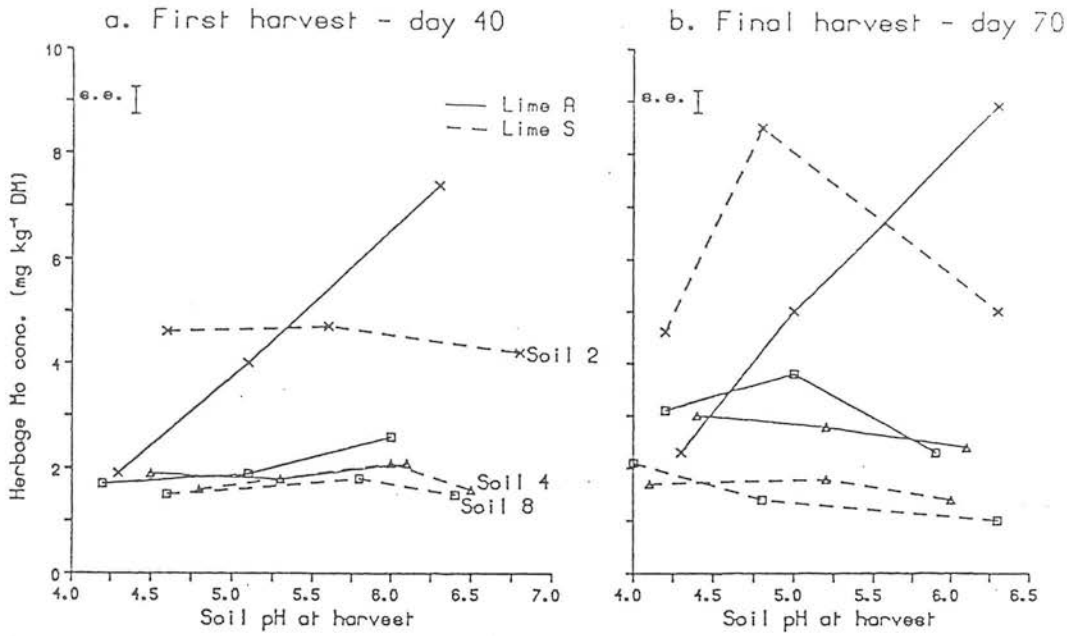

Figure 3.23: Herbage Mo concentration (mg kg^{-1} DM) of PRG grown in soils 2, 4 and 8 to which lime had been applied either to all the soil or to the surface layer (top 30%) of soil.

Herbage Mo concentrations of PRG grown in soils 4 and 8 were not generally significantly affected by lime addition, but for treatment Lime A in soil 2 at the first and second harvest herbage Mo content significantly increased: and although overall at both harvests the Lime S treatment did not significantly affect herbage Mo there was a large increase followed by an equivalent sized decrease between lime levels 1 to 2 and 2 to 3 respectively, at the second harvest. Therefore for soil 2 at the first harvests at lime level 3, Lime A produced higher herbage Mo concentrations than Lime S (there was very poor growth in this treatment) and at lime level 1 Lime A gave lower concentrations than Lime S; the effect of application at lime level 2 is confused by the very different pHs but it appears that method S gave higher herbage Mo concentrations. At the second harvest lime levels 1 and 3 act as at the first harvest and at lime level 2 application method S definitely produced higher herbage Mo contents (see also Table A3.30).

Sulphur - Part A

PRG grown in soil 2 contained more S than WC at all lime levels at both harvests; for lime levels 1 and 2 the size of the difference was 2 to 3 times greater at the second harvest than at the first. For PRG grown in soil 4 the S% was significantly greater than that of WC, at lime level 2 at the first harvest and at lime level 3 at the first and second harvest, all other differences were not significant. In contrast to the other two soils PRG grown in soil 8 contained less S than WC at all lime levels at both harvests (except lime level 3 at the first harvest), the size of the difference was similar at both harvests (Table A3.31).

In PRG the effect of lime additions was always to increase the S concentration of the herbage such that both the overall (L1 to L3) and between lime level differences were significant, the increases were less marked at the second harvest (as found in Experiment 1) (Figure 3.24). WC grown in soil 8 responded to lime additions in the same way as PRG, increased contents, as did WC in soil 2 at the second harvest; and although overall the S content of WC at the first harvest in this soil also increased significantly, the effect of the individual lime additions was not significant. The content of S in WC grown in soil 4 was unaffected by the lime addition from level 1 to level 2 at either harvest whilst the addition from level 2 to 3 led to increased content at the first and decreased content at the second harvest.

Although the spread of S contents found in the herbage was the same at both harvests (0.18%) most values lay between 0.17 and 0.25% at the first and 0.08 and 0.20% at the second, that is the S content of the herbage decreased during the experiment (as in Experiment 1). The decrease was significant in all treatments except soil 2 lime levels 1 and 2 growing PRG.

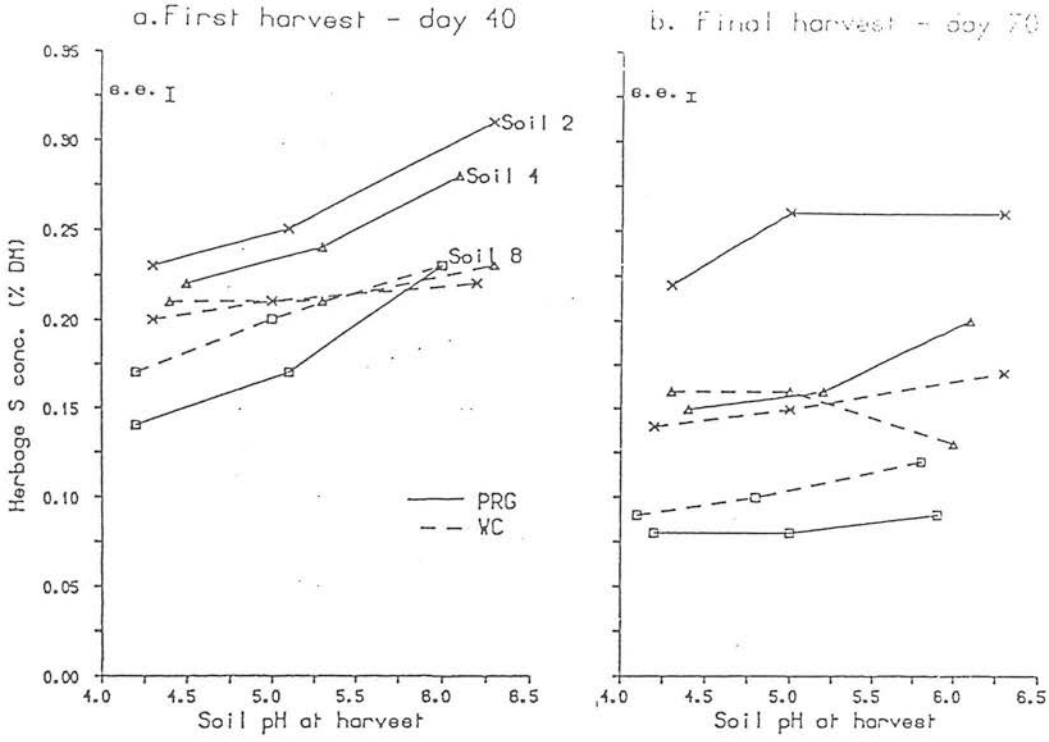

Figure 3.24: Herbage S concentration (% DM) of PRG and WC grown in soils 2, 4 and 8 to which lime had been added.

Sulphur - Part B

The S content of PRG exhibited the same trends with time as in Part A, i.e. a reduced effect of pH and a lower range of contents, although in Part B the spread was wider. The data for the second harvest is presented in Table A3.21; the method of lime application had no significant effect on S% at the second harvest despite the significant effect in soil 2:- Lime S < Lime A.

At the first harvest there was an effect of lime application method but this was confused with the effect of lime addition and the fact that soil pHs were not identical at the different levels in the Lime A and S pots. In soil 2 lime application method S led to higher S% in the herbage at all levels and whilst the effect was probably genuine at level 1, it was

probably not genuine at levels 2 and 3 because of the pH differences. Likewise in soil 8 there was probably no difference between the two methods of application once pHs were considered despite Lime S causing significantly higher S% at levels 2 and 3 than Lime A (possibly the effect at level 3 was genuine). For soil 4 the methods of application had no significant effect on S% in the herbage (Figure 3.25).

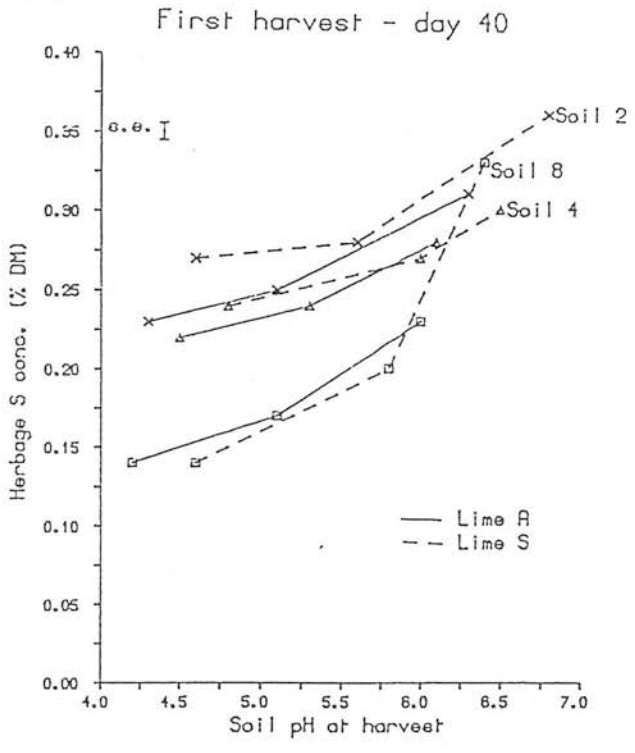

Figure 3.25: Herbage S concentration (% DM) of PRG grown for 40 days in soils 2, 4 and 8 to which lime had been applied either to all the soil or to the surface layer (top 30%) of soil.

Overall (L1 compared to L3) there was a significant increase in herbage S% with lime application in all treatments. The increase was also significant between levels 1 and 2, and 2 and 3 in all soils except for soil 2 and 4 at Lime A levels 1 to 2 and soil 2 at Lime S levels 1 to 2.

Calcium - Part A

The Ca% of WC was approximately 3 times that of PRG, mean 1.64% compared to 0.58%; the species difference in Ca content is normally found to be of this order (e.g. Whitehead, 1972) (Table A3.22).

The effect of lime addition on the herbage Ca% varied greatly with soil and species (Figure 3.26). The Ca% of both species grown in soil 2 increased with lime addition both overall (L1 to L3) and between levels at the first harvest. At the second harvest for this soil lime application had not affected the Ca content of PRG whilst for WC there was an increase between level 1 and 2 followed by a larger decrease between levels 2 and 3 leading to an overall decrease. For herbage grown on soil 4 the addition of lime produced decreased Ca contents in WC, and no significant effect in PRG. WC grown in soil 8 had increased Ca content with lime addition, both overall and between levels (except L2 to L3 at the first harvest) whilst PRG had decreased contents at the first harvest (the decrease occurred between L1 and L2) and at the second harvest (between L2 and L3).

When the two species were statistically analysed together lime addition had a very highly significant effect at both harvests. But when the two species were analysed separately (because of the very different contents and hence unequal variances between the species) lime addition was found to have no effect on the Ca content of PRG at either harvest but for WC there were still effects - highly significant at the first and very highly significant at the second harvest.

The Ca concentration of both WC and PRG altered during the course of the experiment but there was no consistent pattern (Table A3.22).

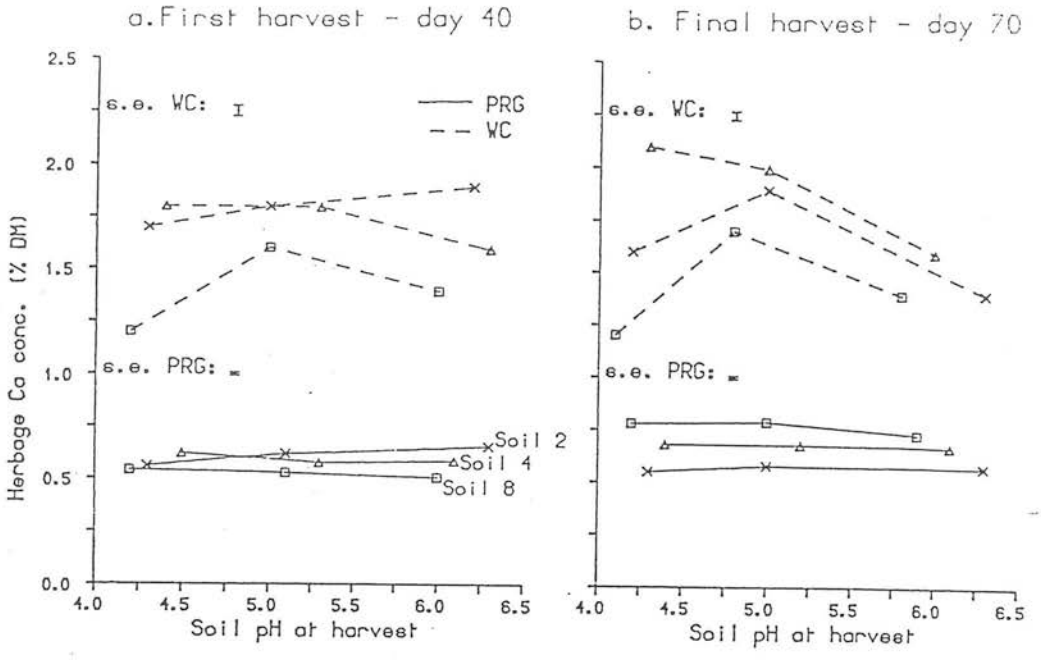

Figure 3.26: Herbage Ca concentration (% DM) of PRG and WC grown in soils 2, 4 and 8 to which lime had been added.

The lack of effect of lime application on the Ca concentration of PRG, in contrast to the apparently logical effect in WC of increasing rates of lime application leading to increased Ca concentrations, could be because of the lower Ca need of the monocot PRG than the dicot WC. That is, whereas PRG is supplied with sufficient Ca at the lowest lime level and does not exhbiit luxury uptake, WC, with a greater Ca requirement, either cannot attain sufficient Ca at any lime level or exhibits luxury uptake of Ca. This hypothesis is backed up by the fact that plant Ca content is controlled more by genetic factors than the soil supply (Mengel and Kirkby, 1982). The higher Ca^{2+} requirement of dicotyledons (such as WC) compared with monocotyledons (such as PRG) is believed to be because dicots have a higher CEC both in their roots and elsewhere (Crooke and Knight, 1971) - hence it would have been interesting to have analysed the plant roots both for CEC and for Ca% to see if there was a species difference.

Calcium - Part B

The Ca% was greatest in plants grown in Lime A pots than Lime S pots at all lime levels and in all three soils (the difference was not significant for soil 2 lime level 1) and was largest at lime level 3 (Figure 3.27).

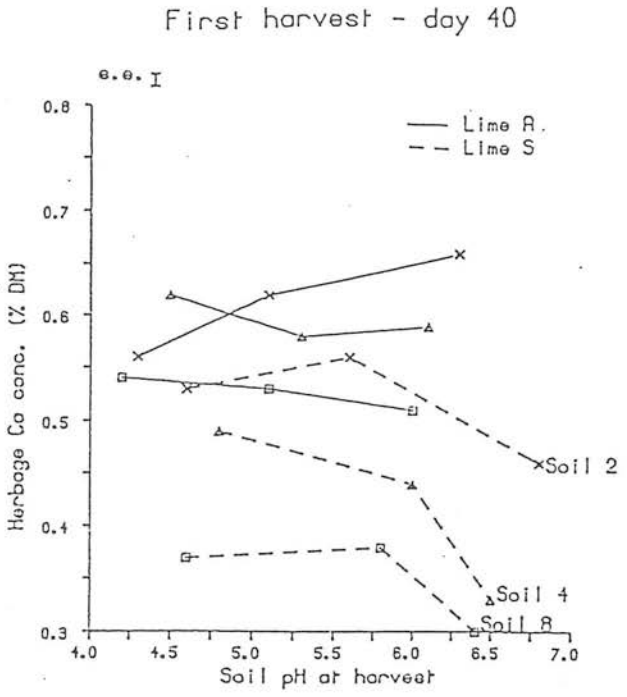

Figure 3.27: Herbage Ca concentration (% DM) of PRG grown for 40 days in soils 2, 4 and 8 to which lime had been applied either to all the soil or to the surface layer (top 30%) of soil.

The effect of lime addition was more consistent than in Part A. PRG grown in Lime S pots always had decreased overall Ca content with lime addition (L1 compared to L3), but for soils 2 and 8 at both harvests there was no significant difference between levels 1 and 2. Lowered overall herbage Ca content was also observed with Lime A for soil 4 at the first harvest and soil 8 at both harvests. There was

no significant overall effect of lime on herbage grown in soils 2 and 4 with Lime A at the second harvest despite an increase in soil 2 between lime levels 1 and 2. For herbage at the first harvest grown in soil 2 with Lime A there was a significant increase in concentration which all occurred between lime levels 1 and 2.

The content of Ca in the herbage was higher at the second harvest than the first for all lime levels with both methods of application on soils 4 and 8 (the difference was not significant for soil 4 Lime S level 3) and lower for all lime levels with both methods of application in soil 2 (the difference at Lime A level 1 was not significant).

Although lime application did not affect the Ca% of PRG when the lime was applied throughout the soil (as also observed in Part A) when the lime was surface applied (top 30% of soil) increasing the amount of lime applied led to decreased plant Ca contents. Ca^{2+} absorption can only occur at young root tips (Robards *et al.*, 1973) so two possibilities arise as to why surface applied lime gave rise to lower plant Ca% than lime applied throughout the soil; either when the lime was concentrated, as in the Lime S pots, the young root tips were damaged so reducing Ca^{2+} uptake; or, that since the Ca^{2+} ions were concentrated in the surface layers once the roots had grown below this layer less Ca could be taken up. This latter possibility would seem to be favoured since the effect was more pronounced at the second harvest when the roots were no longer confined to the highly limed top layer of soil; however, since at both harvests the effect was more marked between lime levels 2 and 3 than levels 1 and 2 the former possibility could be the predominant cause.

2.3 Experiment 3: *An investigation into the effect of nitrogen fertilizer on the content of copper, molybdenum and sulphur in perennial ryegrass grown in soils 2 and 8.*

The basic experimental design was:

2 soils (2, 8) x 3 N-levels (0, 40, 80 kg N ha^{-1}) x 3 forms of N (NH_4^+-N, NO_3^--N, urea) x 4 replicates growing PRG for 42 days when a destructive harvest was taken. (Full details are given in Chapter II, Section 4.1.)

Where the data is presented graphically, plots of the measured variable against the amount of nitrogen applied (as kg N ha^{-1}) are given. The data for the urea treatments are not presented in the main text because the results followed the same pattern as those for NH_4^+-N, presumably since once added to the soil the urea was very rapidly hydrolysed to NH_4^+-N via the enzyme urease (Mohammed *et al.*, 1984). The treatment means for urea, together with those for the NH_4^+-N and NO_3^--N treatments are given in Appendix 3.3.

(a) Soil analysis

pH

At the start of the experiment all treatments had a soil pH of 5.5 (as intended), except soil 2 with NH_4^+-N at 80 kg N ha^{-1} where the pH was 5.7 (Table 3.5).

At the conclusion of the experiment the pH of all treatments had decreased by at least half a pH unit, such a decrease was also observed during Experiment 2 but not Experiment 1. The pH for all soil 2 treatments was now 5.0, except for NH_4^+-N at 80 kg N ha^{-1} which had a pH of 4.9, i.e. a decrease of 0.8 units. The pHs of the NO_3^--N pots in soil 8 were also 5.0, whilst those of the control (no N) and NH_4^+-N pots were 4.9.

Table 3.5: pH for soils 2 and 8 before and after 42 days growth of PRG with and without additional N, applied as NH_4^+-N and NO_3^--N.

Soil	Sampling day	pH				
		No N	NO_3^--N		NH_4^+-N	
			†40	80	40	80
2	0	5.5	5.5	5.5	5.6	5.5
	42	5.0	5.0	5.0	5.0	4.9
8	0	5.5	5.5	5.4	5.5	5.5
	42	4.9	5.0	5.0	4.9	4.9

s.e.: day 0 no nitrogen = 0.04, plus nitrogen = 0.05
day 42 no nitrogen = 0.08, plus nitrogen = 0.02

†40, 80: kg N ha^{-1} applied.

The decline of soil pH with time in glasshouse pots experiments has been discussed on page 109, however, it would be expected that the form of N-fertilizer applied would modify this. NO_3^--N application would be expected to raise soil pH during the course of an experiment, both because of denitrification and plant excretion of HCO_3^- ions when NO_3^- ions are taken up (as observed by Jarvis and Robson, 1983), but a decrease in soil pH was noted. Decreases of similar size were also noted when no nitrogen or NH_4^+-N was applied, so there was no evidence of NH_4^+ acidifying the soil more than NO_3^- or no N. The possibility that there were differences in soil pH had the rhizosphere soil rather than bulk soil been analysed, must not be dismissed.

(b) Herbage analysis

Dry matter production

In both soils DM production increased with N application, NO_3^--N gave slightly greater production than NH_4^+-N. The amount of production was greater in soil 8 than soil 2 at all N-levels but particularly in the control pots (no N applied) where the production in soil 8 was four times that in soil 2 (Figure 3.28 and Table A3.24).

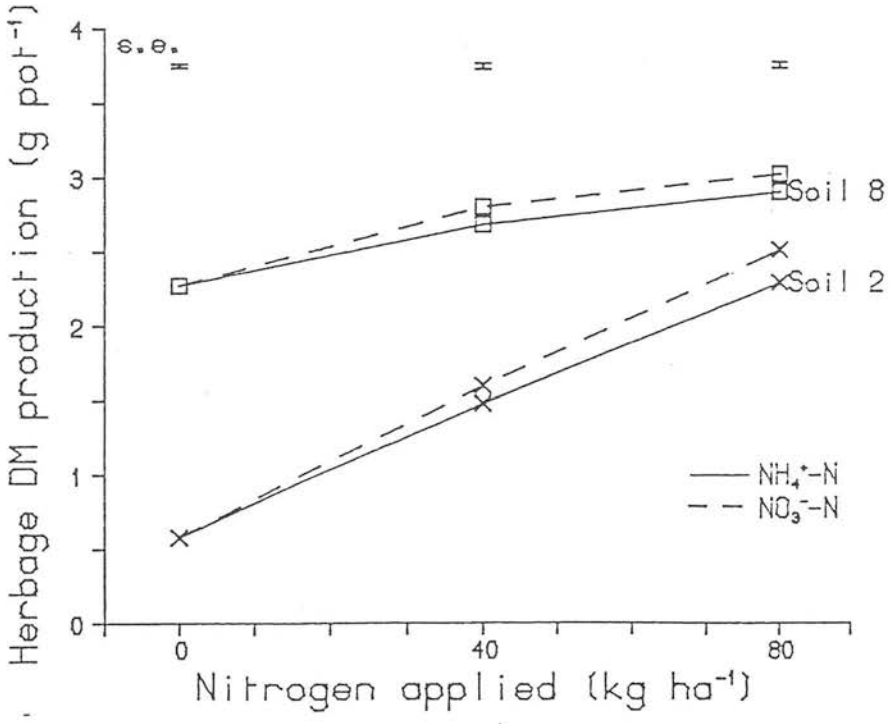

Figure 3.28: Herbage DM production (g pot^{-1}) of PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N or NO_3^--N.

Copper

PRG growing in soil 2 had Cu concentrations three times greater than those of PRG growing in soil 8 - similar results were found in both previous experiments. The addition of N had no effect on plant

Cu concentration in any treatment, except for soil 2 with NO_3^--N at 40 kg N ha^{-1} where a small decrease in herbage Cu concentrations was observed (Figure 3.29 and Table A3.25).

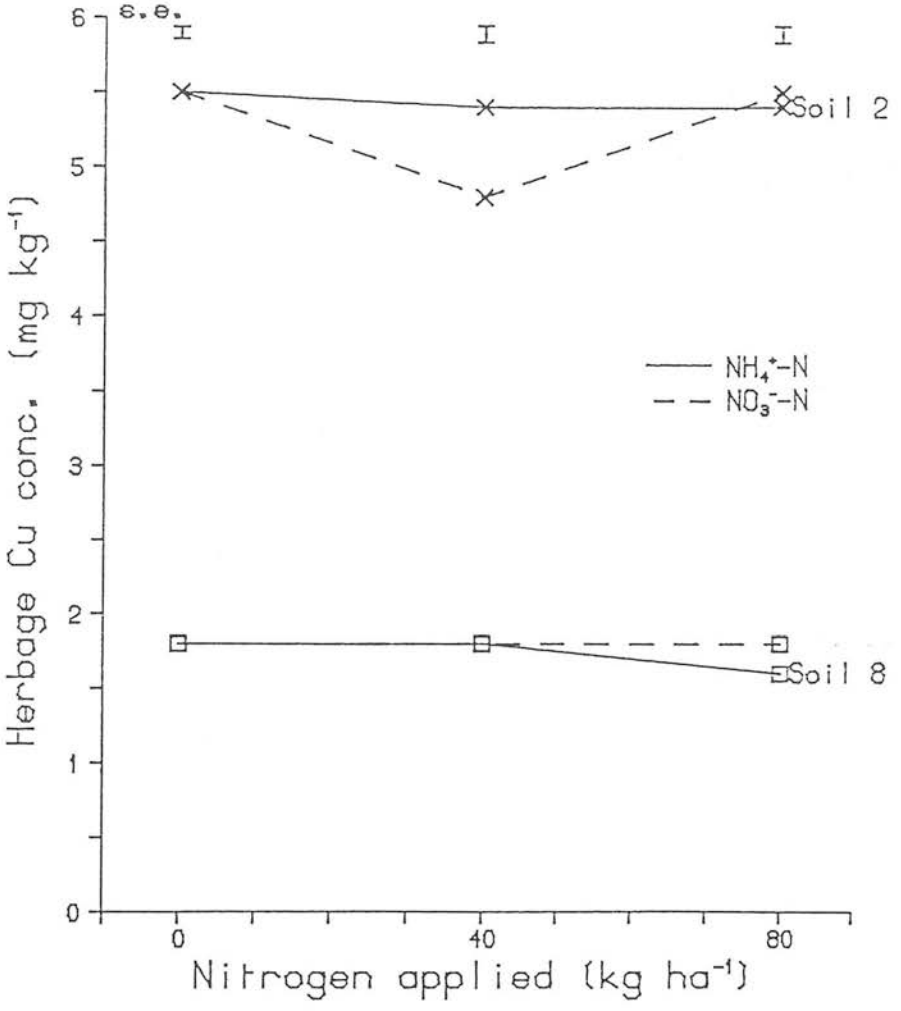

Figure 3.29: Herbage Cu concentration (mg kg^{-1} DM) of PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N or NO_3^--N.

Molybdenum

The addition of N had no effect on the concentration of Mo in PRG growing in soil 8 but led to a 50% decrease in Mo content of PRG growing in soil 2 (Figure 3.30 and Table A3.26).

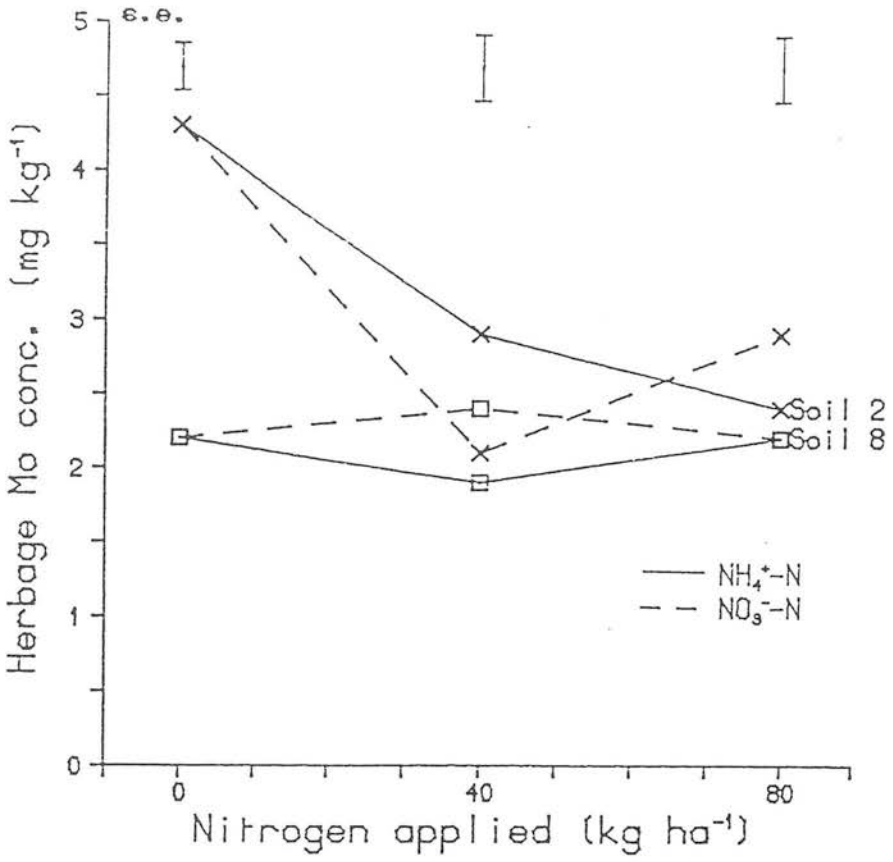

Figure 3.30: Herbage Mo concentration (mg kg^{-1} DM) of PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N or NO_3^--N.

Sulphur

There were large between-soil differences in the S content of PRG, plants grown in soil 2 contained two to three times as much S as plants grown in soil 8 - this was also found in other experiments. The application of NH_4^+-N to soil 8 had no effect on the PRG S concentration whereas the application of NO_3^--N led to a small decrease - all the significant decrease occurred with the first level of N application. The application of N to soil 2 caused large decreases in PRG S content; for NO_3^--N both N additions caused decreases, whilst for NH_4^+-N all

the significant decrease occurred with the first level of application (Figure 3.31 and Table A3.27).

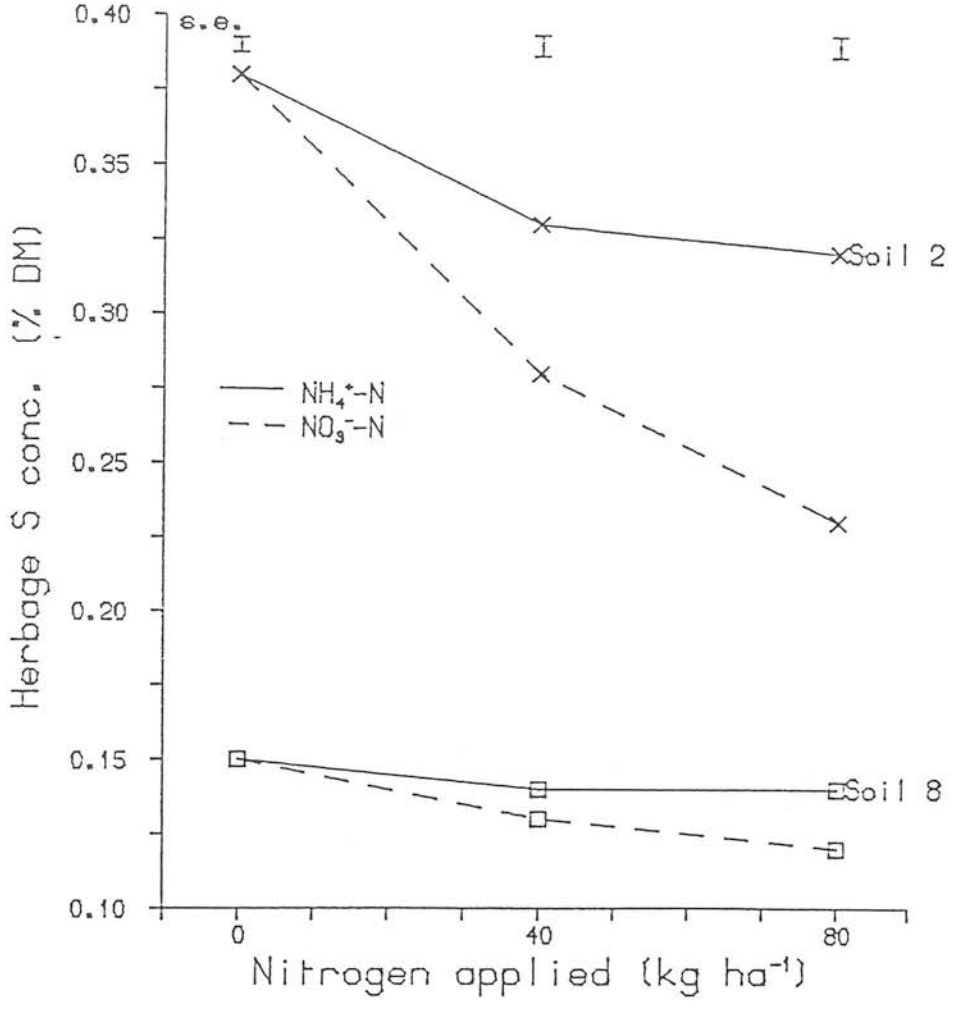

Figure 3.31: Herbage S concentration (% DM) of PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N or NO_3^--N.

Nitrogen

The concentration of N was higher in PRG grown in soil 8 than soil 2. The addition of N increased the N content of the PRG: for soil 2 the increase only occurred with the second N addition (40 to 80

kg ha^{-1}), whilst for soil 8 the increase occurred with both N additions and varied with N-form - the addition of NH_4^+-N caused a greater increase in plant N per cent than the addition of NO_3^--N (Figure 3.32 and Table A3.28).

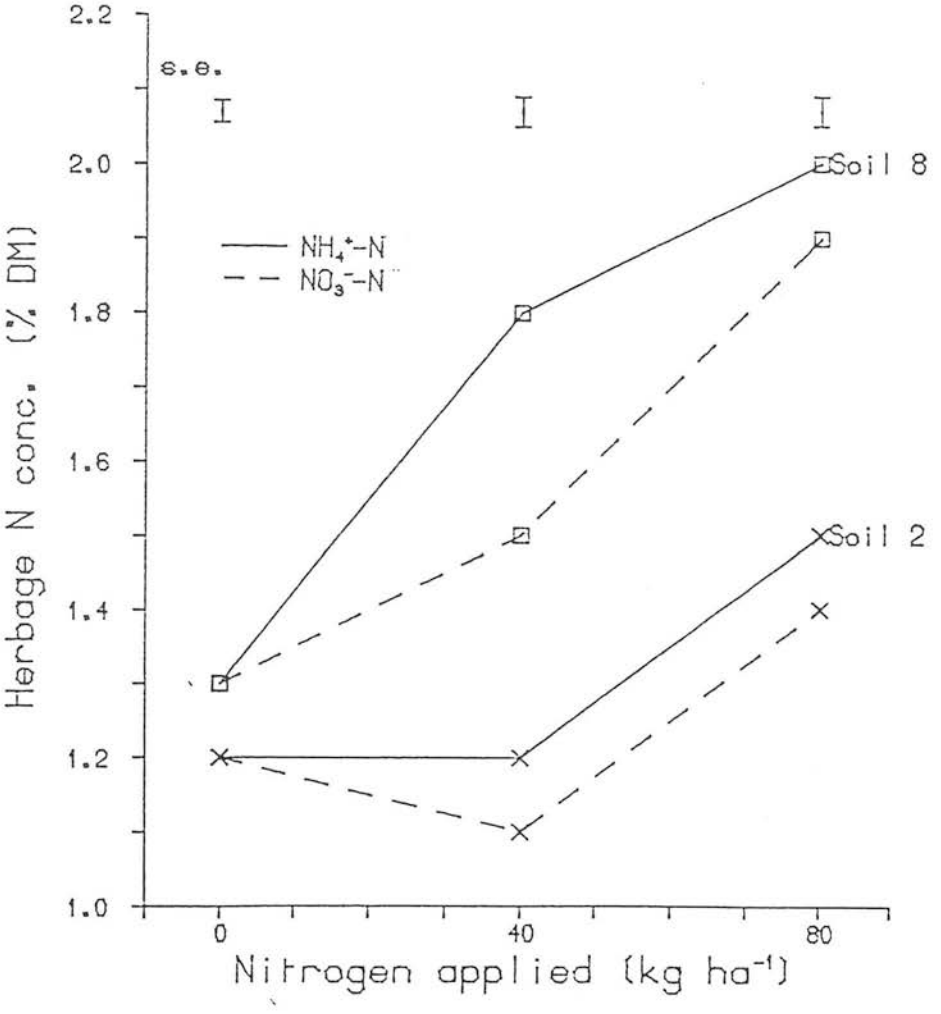

Figure 3.32: Herbage N concentration (% DM) of PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N or NO_3^--N.

The greater increase in PRG N concentration for plants grown in soil 8 rather than soil 2 is consistent with the smaller response of plant DM production in soil 8 (Figure 3.28) and the lower initial N

content of soil 2 (Table 3.2). That is, soil 2 could naturally supply only just enough N to meet the requirements of PRG, so at 40 kg N ha^{-1} plants had similar N concentrations to when no N was applied since the extra DM production matched the additional N supply. For plants growing in soil 8 N supply was always adequate so "luxury" uptake (uptake of more of a nutrient than is necessary to supply a plant's requirement) occurred at both N application rates whereas for soil 2 this only occurred with 80 kg N ha^{-1}.

The "luxury" uptake of N observed in soil 8 did not seem to interact with the trace-element concentrations in PRG as soil 8 exhibited less response to N fertilization with regard to PRG concentrations of Cu, Mo and S than soil 2 (see Chapter IV, Section 4.2).

2.4 Experiment 4: *An investigation into the effects of liming and phosphorus fertilization on the content of copper, molybdenum and sulphur in perennial ryegrass and white clover grown in soils 2 and 8.*

The basic experimental design was:

2 soils (2, 8) x 2 pH (lime levels 1, 2 - L1, L2) x 3 P-levels (1, 2, 3 - P1, P2, P3) x 2 species (PRG, WC) x 4 replicates; with 2 sequential harvests on days 36 (T1) and 52 (T2). (Full details are given in Chapter II, Section 4.1.)

The results are presented mainly as plots of the variable against the amount of P applied as kg P ha^{-1}, except for pH which is plotted against time. Although the results of both the initial and final soil analyses are given in Appendix 3.4, it is the final soil analyses which are considered in the following sections unless the two sets of analyses were very different. For the herbage results only those for the first harvest are considered in the main body of the text, as generally there was little difference between the results of the two harvests. Also, soil pH was unaffected by P-level at the first harvest so it is therefore valid to plot the results against P-applied but at the second harvest soil pH was significantly affected by P-level so such an approach is not valid. Major differences between the two harvests are mentioned briefly and the results for the second harvest are presented in Appendix 3.4.

There were two lime additions made in this experiment, that to raise the natural pH (*c.* 3.5) to *c.* 5.0 (lime level 1) and that to raise the pH to *c.* 6.5 (lime level 2). Since it was known that production of PRG would be very poor at natural pH (see Experiment 1) and strongly suspected that WC growth would also be very poor because of an inability to nodulate properly (Munns, 1978), unlimed soil was not used in the

experiment, therefore liming comparisons are between two limed soils, not the effect of liming *per se*.

(a) Soil analyses

pH

The application of the calculated quantities of lime to the two soils successfully gave initial soil pHs of 4.9 - 5.0 and 6.5 (lime levels 1 and 2 respectively). Soil 8 had higher pHs than soil 2 at all three sampling times, but the difference was very small (generally ≤0.1 unit) (Table 3.6)

Table 3.6: pH for soils 2 and 8 in which PRG or WC were grown at two lime levels and three P-levels.

Soil	Lime level	Sampling time†	pH					
			WC			PRG		
			[2]P1	P2	P3	P1	P2	P3
2	1	0	4.9	4.9··	4.8	4.9	4.9	4.9
		1	4.7	4.6	4.6	4.6	4.6	4.6
		2	4.5··	4.5	4.4	4.5	4.5	4.5
	2	0	6.4	6.6	6.5	6.5	6.5	6.5
		1	6.0	5.8	6.0	6.0	6.0	5.9
		2	5.7	5.8	5.8	5.9	5.9	5.9
8	1	0	4.9	5.0	5.0	4.9	5.0	5.0
		1	4.8	4.8	4.6··	4.6	4.6	4.6
		2	4.6	4.6	4.5	4.5	4.5	4.5
	2	0	6.4	6.5··	6.5··	6.5	6.6	6.5
		1	6.2	6.3··	6.2	5.9	5.9··	5.9
		2	6.0	6.0	5.8··	5.8	5.8	5.8

s.e.: day 0 = 0.04; Harvest 1 = 0.04; Harvest 2 = 0.02

†0, 1, 2: day 0, start of experiment, and days 36 and 52, first and second harvests.

[2]P1, P2, P3: phosphorus levels 1, 2 and 3 equivalent to 24, 48 and 96 kg P ha^{-1}.

The plant species growing in the soil did not affect soil pH except at the first harvest where the pHs of soil 8 in which WC had been grown were higher than those where PRG had been grown by 0.2 units. This contrasts with the results of Experiment 2A.

The application of P fertilizer had no significant main effect on soil pH at the first harvest, although it had a very highly significant but inconsistent effect at the start and conclusion of the experiment. However, despite being significant the effects were small (see Table 3.6) ≤ 0.3 units and will have had an insignificant effect compared to the difference between the two lime levels.

The soil pH of all treatments decreased over the course of the experiment by 0.3 - 0.8 units with the magnitude of the decrease being greater the higher the initial pH and continued throughout the experiment for soils in which WC was grown, whilst for soils in which PRG was grown most of the decrease had occurred by the first harvest. These observations were consistent with those of Experiments 2 and 3, but unlike Experiment 2 the decreases were generally slightly greater in soil 8 than soil 2.

Extractable copper

The concentrations of extractable soil Cu were 6 - 10 times lower in soil 8 than soil 2, soil means of 0.5 and 4.7 mg Cu kg^{-1} respectively at the final harvest. The amount of extractable soil Cu was greater for soil 2 for pots in which PRG had been grown than for pots in which WC had been grown (except for L1 at P2 and L2 at P3 where there was no difference). A species main effect was also present in soil 8 but to a lesser extent ($p < 0.05$) and pots in which PRG had been grown contained more extractable soil Cu for only two treatments (L1 at P1 and P2), all other treatments showed no species effect.

All treatments in soil 2 had greater extractable soil Cu contents with increased lime addition ($p < 0.001$) but for soil 8 the effect was less significant ($p < 0.05$) (Figure 3.33), and only occurred for pots growing WC at P-levels 2 and 3. Similar results were observed in Experiment 1B. P fertilization had no effect on the Cu content of soil 2 but had a highly significant effect ($p < 0.01$) for soil 8 with overall decreases (P1 *cf.* P3) being observed in all treatments, except WC at lime level 2.

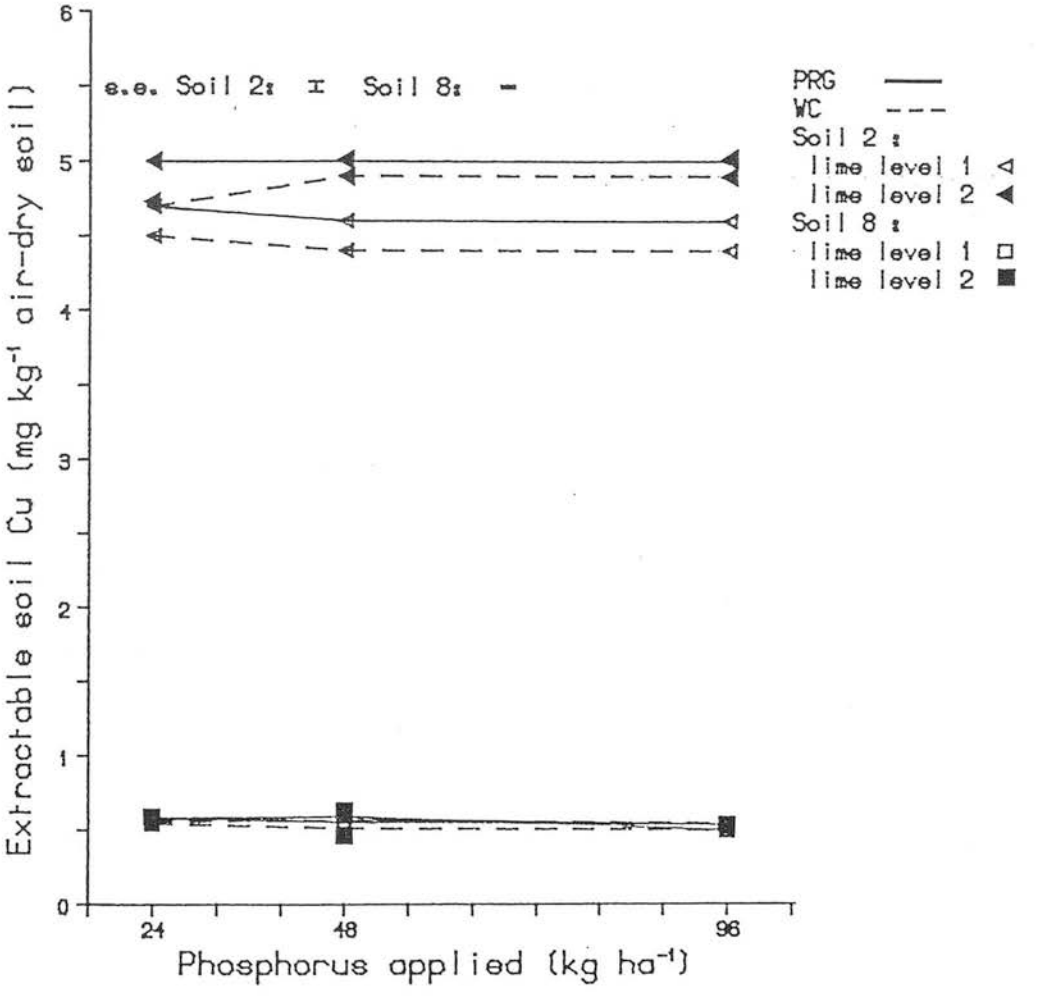

Figure 3.33: Extractable Cu (mg kg^{-1} air-dry soil) for soils 2 and 8 in which PRG or WC were grown for 52 days at two lime levels and three P-levels.

When the soil extractable Cu contents at the final harvest were compared to those at the start of the experiment, it was observed that concentrations in soil 2 had risen by 1.0 - 1.5 mg Cu kg^{-1}, whilst the lower concentrations found in soil 8 were generally unaltered (Table A3.29).

Extractable molybdenum

The content of extractable soil Mo was 1½ to 3 times higher in soil 2 than soil 8 (range 0.19 - 0.39 *cf.* 0.045 - 0.082 mg Mo kg^{-1} air-dry soil). The effect that the species growing in the soil had on Mo content varied with the other factors (soil, lime level and P-level). For soil 2 the species effect only occurred at P-level 1 where for both lime levels soil in which WC had been grown contained more extractable Mo than soil in which PRG had been grown. For soil 8 half the treatments had more soil extractable Mo after WC growth than PRG growth and the other half were not affected (Figure 3.34).

The addition of lime (level 1 to 2) had no effect on extractable soil Mo contents for soil 8, but led to increased contents in soil 2 for all treatments (*c.* 50% increases). (A similar result was observed at the start of the experiment and also in Experiment 1.) By the final harvest P fertilization had no effect on soil extractable Mo content although at the start of the experiment the addition of P had apparently led to increased overall soil Mo in soil 2 at both lime levels, and a decrease for soil 8 at lime level 2 (Table A3.30).

Generally, the amount of soil extractable Mo was unaltered during the course of the experiment for soil 8 whilst decreasing for soil 2.

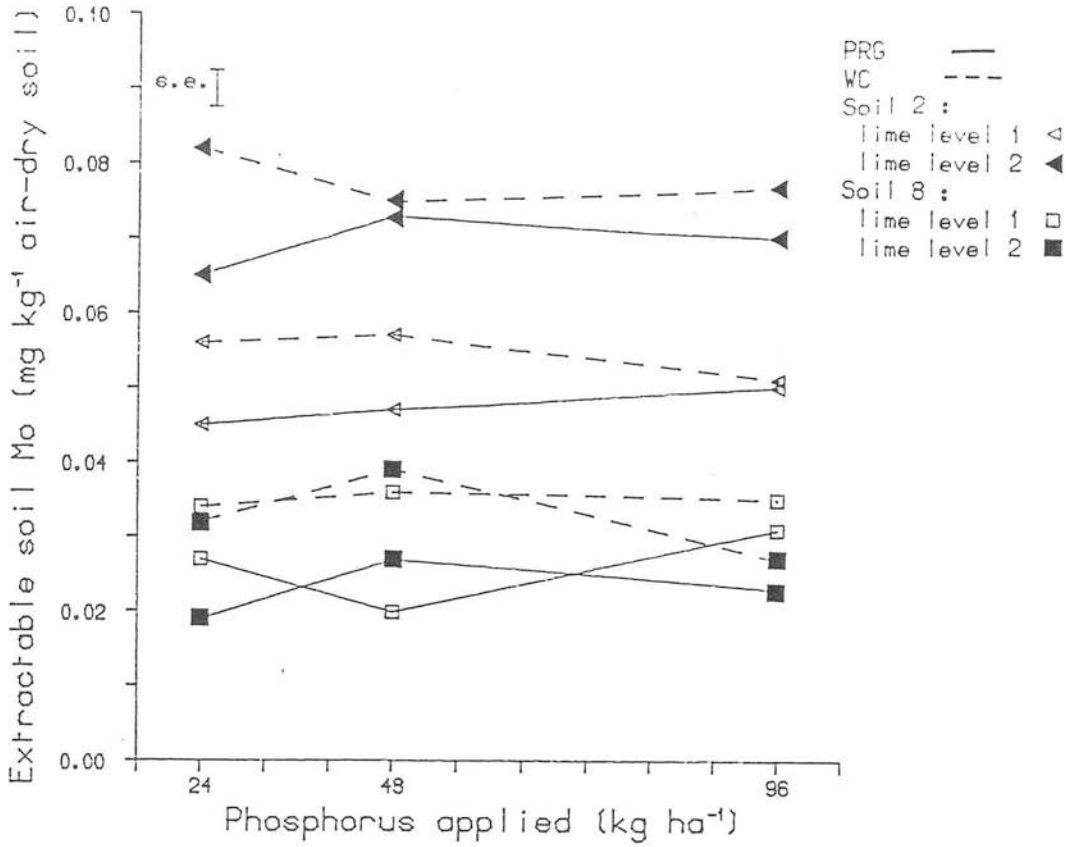

Figure 3.34: Extractable Mo (mg kg^{-1} air-dry soil) for soils 2 and 8 in which PRG or WC were grown for 52 days at two lime levels and three P-levels.

Extractable sulphate-sulphur

The content of extractable SO_4^{2-}-S was greater in soil 2 than soil 8, the size of difference varied with the treatments being applied, for WC at lime level 2 the differences were very small, but was generally in the order of 100%. Soil in which PRG had been grown had a considerably lower SO_4^{2-}-S content at the end of the experiment than soil in which WC had been grown (except soil 8, L1 at P3 where there was no difference), the difference was particularly great for lime level 2 treatments.

The application of lime to a soil led to increased soil SO_4^{2-}-S in all pots in which WC had been grown, but had no effect on the soils in which PRG had been grown (a small increase was observed for soil 2 at P-level 3). This result was in contrast to Experiment 1 (Parts A and B) where PRG was grown and both soils, 2 and 8, exhibited increased soil extractable SO_4^{2-}-S contents with lime addition. P fertilization, like lime addition, led to no alteration in the level of SO_4^{2-}-S extracted from soils in which PRG had been grown but led to decreased contents in soils in which WC had been grown; since the amount of SO_4^{2-}-S extractable before plant growth was unaffected by P-addition this effect seems to be due to WC growth (Figure 3.35).

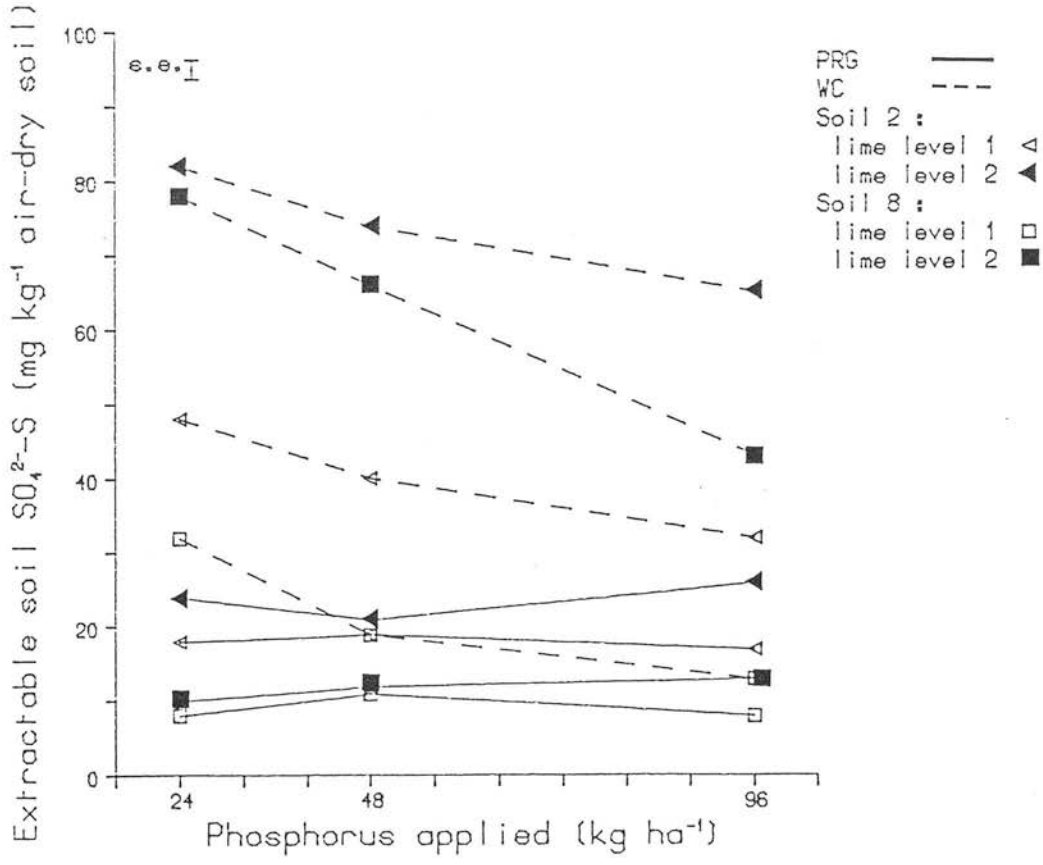

Figure 3.35: Extractable SO_4^{2-}-S (mg kg^{-1} air-dry soil) for soils 2 and 8 in which PRG or WC were grown for 52 days at two lime levels and three P-levels.

From comparing the soil contents of extractable SO_4^{2-}-S at the beginning and end of the experiment (Table A3.31) it can be seen that whereas PRG growth consistently lowered soil SO_4^{2-}-S contents the effect of WC growth was less consistent - decreases, increases or no change being recorded.

Extractable calcium

The amount of extractable soil Ca was greater in soil 8 than soil 2 at each lime level as would be expected since more lime was added to soil 8 than soil 2 to achieve the desired pHs. The plant species which had been grown in the soil had no effect on final Ca content, although there was apparently less Ca in soil 2 at lime level 2 and P-level 3 in which WC had been grown than in this treatment which had grown PRG (Figure 3.36).

The quantities of extractable soil Ca were obviously very different between the two lime levels. The effect of P fertilization was highly significant ($p < 0.01$) but had no overall (P1 compared to P3) effect on soils in which WC had been grown. There was also no effect of P-addition on soil Ca content where PRG had been grown for soil 2, but for soil 8 there were overall increases in Ca content at both lime levels.

From a comparison of the final soil Ca contents with the initial contents (Table A3.32) it is seen that there was generally no difference in soil Ca levels at the two sampling times.

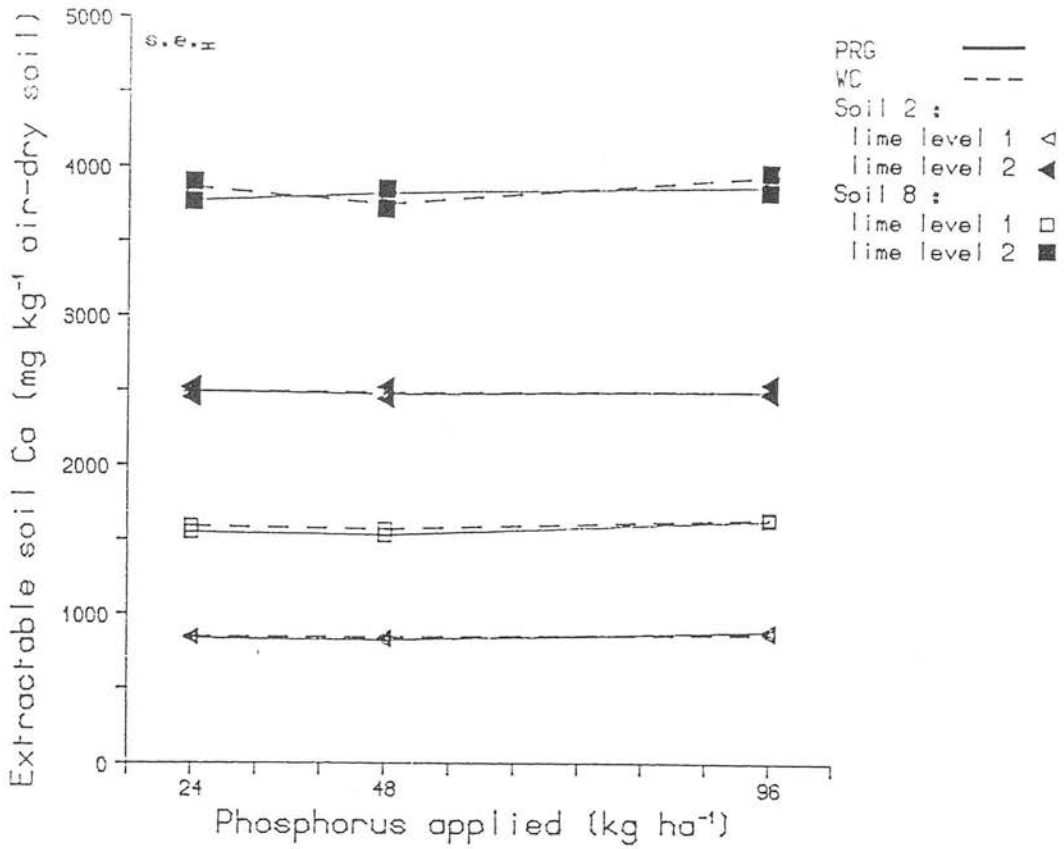

Figure 3.36: Extractable Ca (mg kg^{-1} air-dry soil) for soils 2 and 8 in which PRG or WC were grown for 52 days at two lime levels and three P-levels.

Extractable phosphorus

The content of extractable soil PO_4^{3-}-P was greater in soil 8 than soil 2, the initial (unamended) contents of PO_4^{3-}-P in the two soils were 4.3 and 1.7 mg kg^{-1} oven-dry soil respectively (Table 3.2). There was generally more (20-35% more) PO_4^{3-}-P extractable from soils in which WC rather than PRG had been grown, although for soil 2 at P-level 1 at both lime levels and P-level 2 at lime level 2 there was no difference (Figure 3.37).

The addition of lime had no effect on extractable soil PO_4^{3-}-P levels. The addition of P fertilizer, obviously, led to increased levels of extractable soil PO_4^{3-}-P.

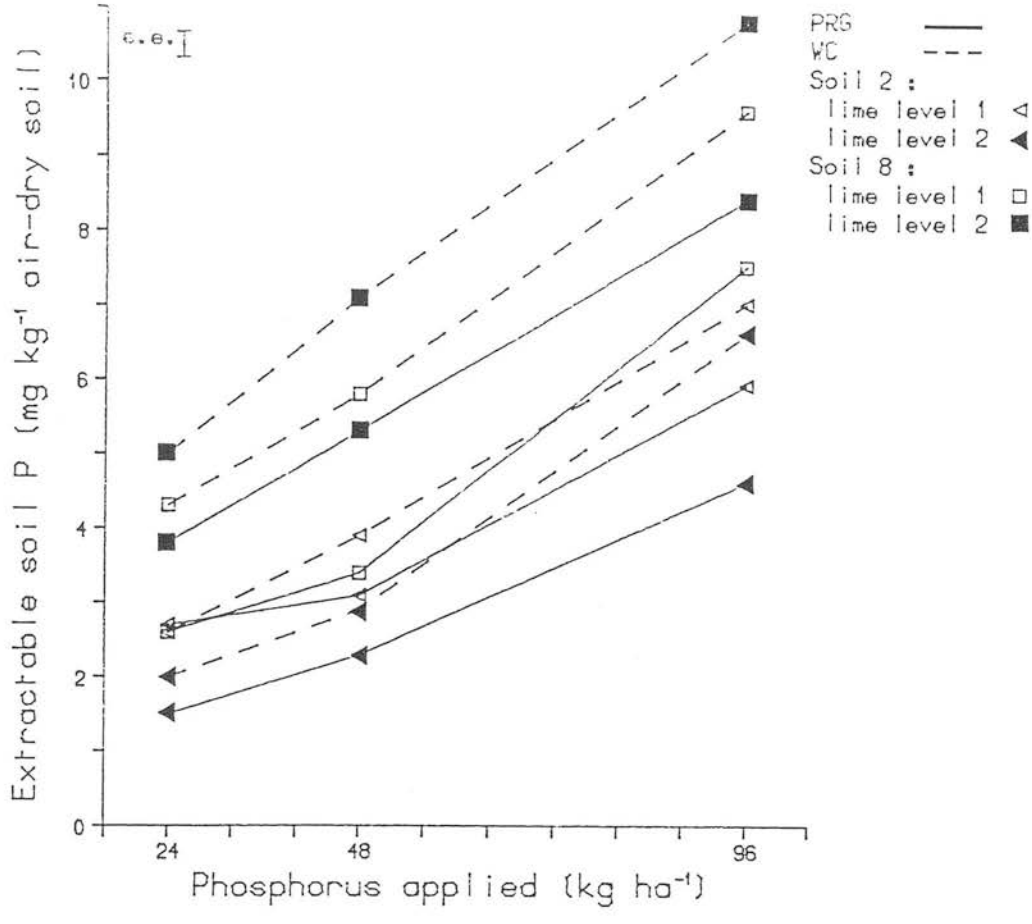

Figure 3.37: Extractable PO_4^{3-}-P (mg kg^{-1} air-dry soil) for soils 2 and 8 in which PRG or WC were grown for 52 days at two lime levels and three P-levels.

For soils in which PRG was grown the levels of extractable PO_4^{3-}-P decreased between the start and conclusion of the experiment except for soil 2 lime level 1 at all P-levels and at lime level 2 P-level 1. For soils in which WC was grown there was no difference between soil PO_4^{3-}-P contents at the two sampling times (except for a small decrease in soil 8 at P3 and soil 2 at L2 and P2) (Table A3.33).

(b) Herbage analyses

Dry matter production

There was a soil effect on dry matter production at the first ($p < 0.001$) and second ($p < 0.05$) harvest; plants grown in soil 8 sometimes produced slightly more herbage than those of the same treatment in soil 2. At both harvests PRG produced more herbage than WC, although the magnitude of the difference was smaller at the second harvest (approximately 1½ times more production for PRG compared to 5½ times more), because PRG production had decreased whilst that of WC had increased (Table A3.34).

Lime addition had no effect on herbage production at the first harvest, although it apparently decreased WC production in soil 8 and increased PRG production in both soils. But at the second harvest lime addition had affected herbage production increasing DM production of PRG in both soils and of WC at P-level 3 in both soils. There was no difference in production for WC in soil 2 at lime level 1 and P-levels 1 and 2 and decreased production in these treatments on soil 8 (Figure 3.38).

P-addition (P1 *cf.* P3) increased herbage production of both WC and PRG at the first harvest (although not for PRG grown in soil 8 at lime level 1), but only that of WC at the second.

The overall impression gained from these results is that P-addition tended to increase DM production (except PRG at the second harvest) and that whereas for PRG, liming also increased production (Al toxicity was overcome) for WC the interaction between lime x phosphorus varied with soil, species and harvest, butl generally led to decreased production. It has been proposed (e.g. Davis, 1981) that the reduced growth of

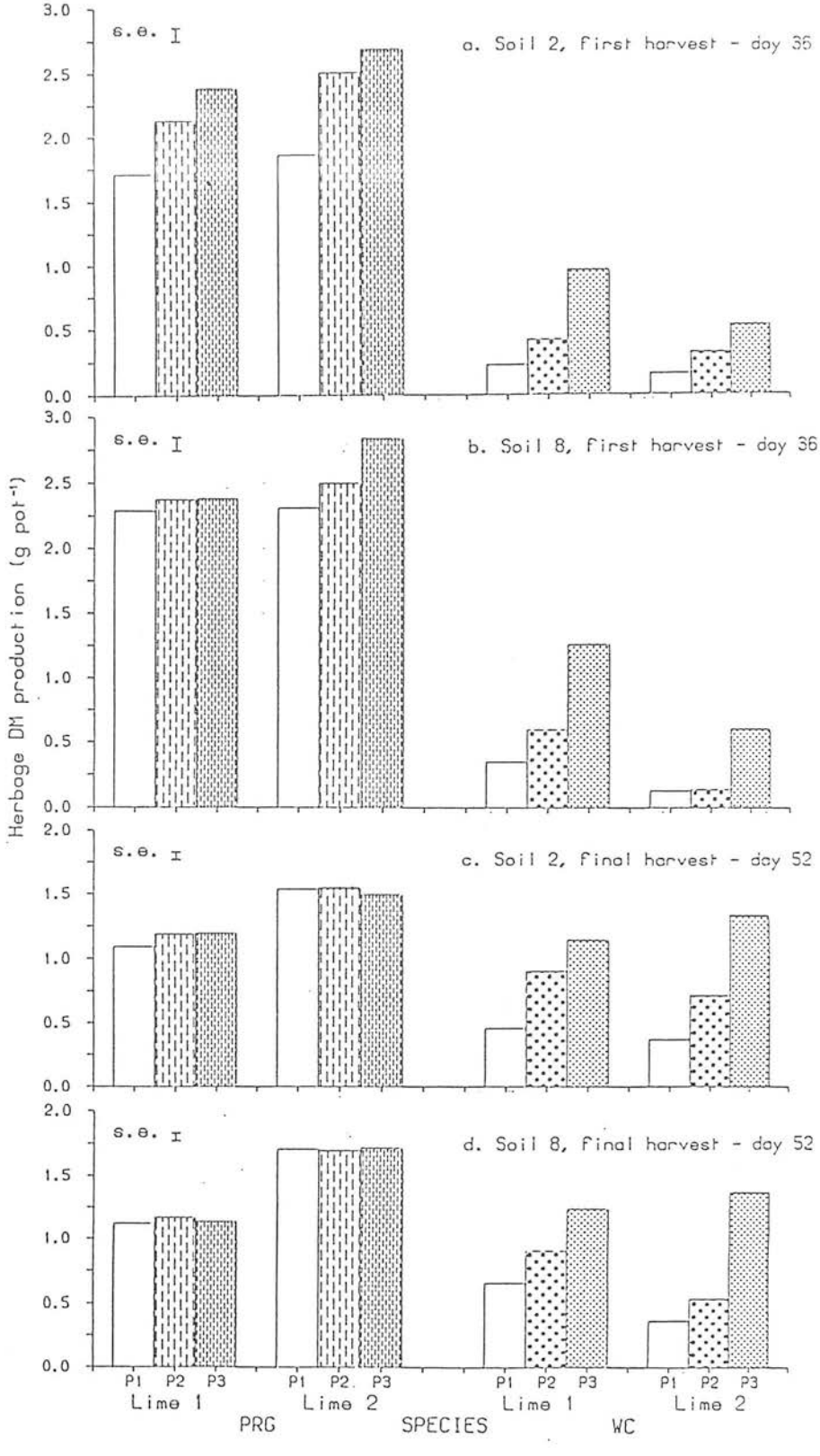

Figure 3.38: Herbage DM production (g pot^{-1}) for PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

legumes brought about by liming is due to an induced P deficiency; and the results presented here support this view since by the time the clover plants were established (the second harvest) although WC growth in both soils was poorer at the lowest two P-levels at lime level 2 than at lime level 1, it was greater at lime level 2 for P-level 3.

Copper

The copper concentration in herbage grown in soil 2 was approximately 2 to 4 times greater than that grown in soil 8 (as also observed in the other experiments), therefore the analysis of variance was carried out separately for each soil. For soil 2 the species effect was very highly significant ($p < 0.001$); WC contained more Cu than PRG at P-levels 2 and 3 at both lime levels, but at P-level 1 and lime level 1 there was no species difference and at P-level 1 and lime level 2 PRG contained more Cu than WC. For soil 8 the species effect was significant at $p < 0.05$, with there being no difference in Cu content of the two species except at lime level 1 and P-level 1, where WC contained more Cu than PRG.

Lime addition had no effect on the Cu content of herbage grown in soil 8 but for that grown in soil 2 it increased Cu content. The addition of P had a significant effect ($p < 0.05$) in both soils but the effect was inconsistent (Figure 3.39).

Slight differences were found in the pattern of results at the second harvest and the data is presented in Table A3.35.

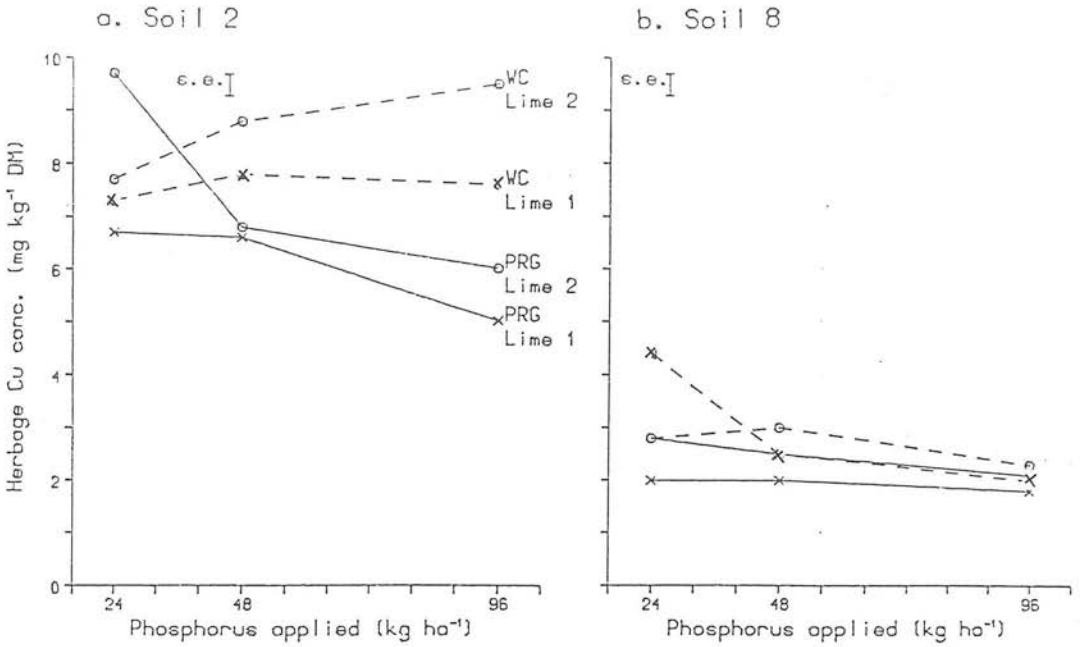

Figure 3.39: Herbage Cu concentration (mg kg^{-1} DM) for PRG and WC grown in soils 2 and 8 for 36 days at two lime levels and three P-levels.

Molybdenum

The effect of soil on herbage Mo content was highly significant ($p < 0.01$) but this effect was due solely to the large differences in WC content at P-levels 1 and 3 at lime level 2 since all other differences were not significant. However, at the second harvest although there was still no effect of soil at lime level 1 or at lime level 2 for PRG, for WC grown at this lime level plants grown in soil 2 contained more Mo than plants grown on soil 8 (Table A3.36).

WC contained more Mo than PRG at lime level 2 for both soils but there was no difference in the Mo contents of the two species at lime level 1.

The addition of lime to the soils led to greatly increased herbage Mo contents (2- to 20-fold increases) for WC grown in both soils, but

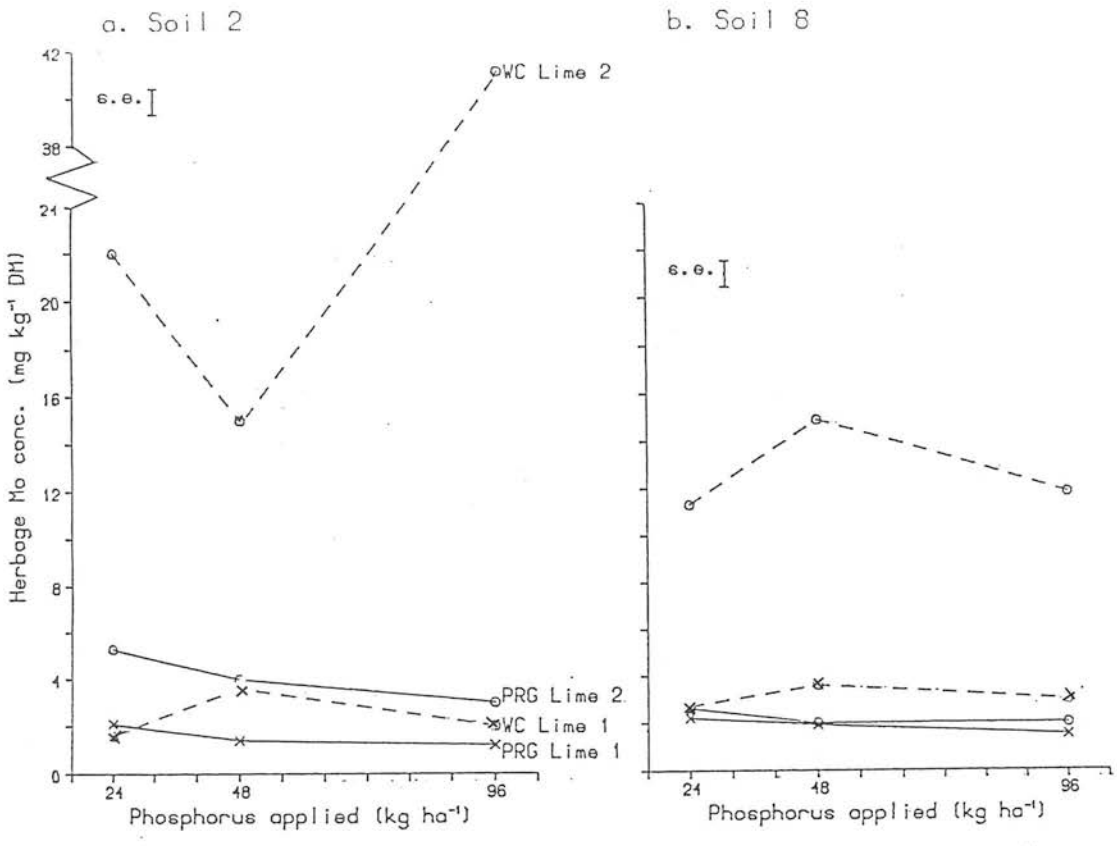

Figure 3.40: Herbage Mo concentration (mg kg^{-1} DM) for PRG and WC grown in soils 2 and 8 for 36 days at two lime levels and three P-levels.

had no effect on the Mo content of PRG grown in soil 8 and increased the Mo content of PRG grown in soil 2 only 2- to 3-fold. The addition of P had no effect on herbage Mo content.

The same pattern of results was observed at the second harvest, although the herbage Mo concentrations were different (Table A3.36).

Sulphur

PRG grown in soil 2 contained approximately ½ to 3 times more S than PRG grown in soil 8; but when WC was grown in soil 2 at lime level 1 and P-levels 1 and 2 and at lime level 2, P-level 1, it contained

less S (c. a fifth) than when grown in soil 8, whilst for the other three treatments (L1 at P3 and L2 at P2 and P3) there was no soil difference (Figure 3.41). WC contained less S than PRG in all treatments, except soil 8 at lime level 1 and P-levels 1 and 2 where it contained more, and soil 8 at P-level 3 where there was no difference. At the second harvest all WC grown in soil 2 contained less S than PRG but for soil 8 WC contained more S than PRG.

The addition of lime led to increased herbage S contents in all treatments except for WC grown in both soils at lime level 1 and P-level 1 and in soil 8 at lime level 1 P-level 2. At the second harvest lime addition did not affect the S content of PRG but it increased that of WC. The effect of P addition was inconsistent (see Figure 3.41).

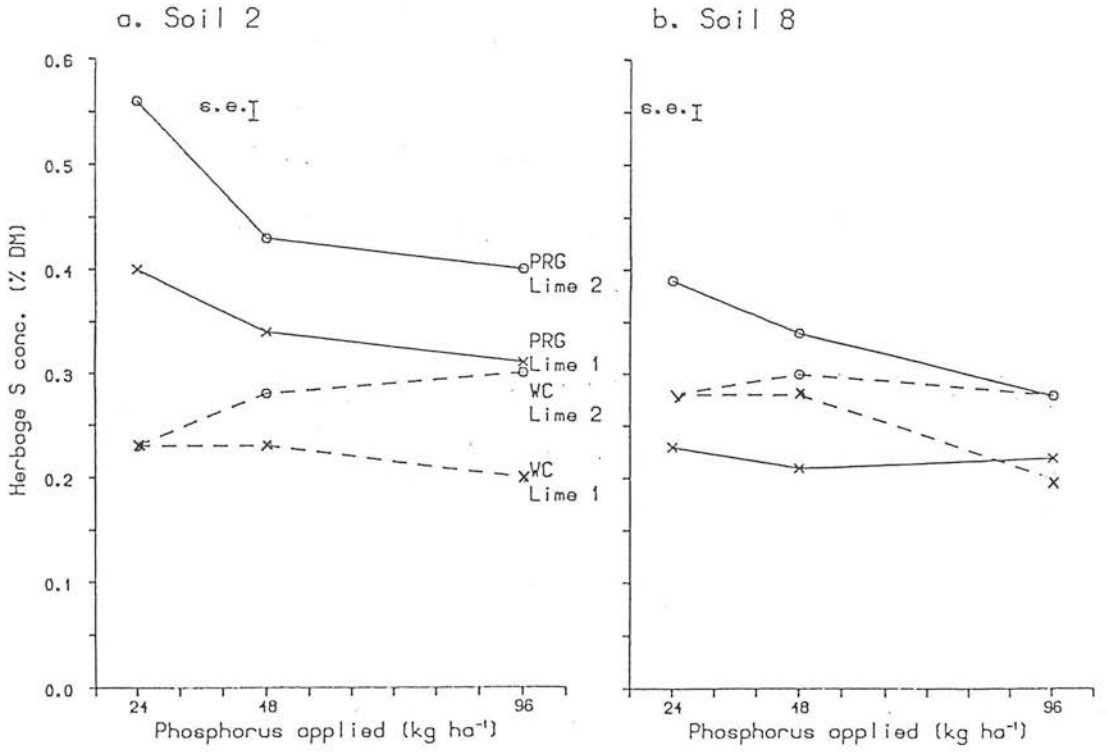

Figure 3.41: Herbage S concentration (% DM) for PRG and WC grown in soils 2 and 8 for 36 days at two lime levels and three P-levels.

In agreement with the findings of other experiments herbage S contents were observed to decrease during the course of the experiment (Table A3.37) and the same basic patterns of results were found at the second as at the first harvest.

Calcium

WC herbage grown in soil 2 contained 25-75% more Ca than WC grown in soil 8, reflecting the difference in soil Ca contents; but there was no difference in the Ca content of PRG grown in the two soils. WC contained approximately 3 times as much Ca as PRG (means of 1.1 and 0.4% respectively), this was also observed in Experiment 2A, so the two species were separated for the analysis of variance. At the second harvest there was an effect of soil on the Ca% of PRG with plants grown in soil 2 containing less Ca than those in soil 8 (Table A3.38).

At the first harvest lime application had no effect on the Ca% of WC, whereas at the second harvest the effect was very highly significant ($p < 0.001$) and for all treatments (except soil 8 at P1) WC plants grown at lime level 2 contained more Ca than those grown at lime level 1 as would be expected from the soil contents. For PRG lime had a small effect on herbage Ca% at the first harvest ($p < 0.05$) manifest as small increases with lime addition in the plants growing in soil 2 at P levels 2 and 3 (Figure 3.42), but there was no effect at the second harvest. This pattern of increases in the Ca% of WC with liming but lack of effect in PRG was also noted and discussed in Experiment 2A.

P-addition did not affect the Ca% of PRG but did affect the Ca% of WC although the magnitude of the effect was modified by soil - large increases were observed in plant Ca% with P-addition to soil 2 but for

soil 8 no increases were observed at lime level 1 and only small increases at lime level 2. (There was no effect of P-addition of the Ca content of either species at the second harvest.)

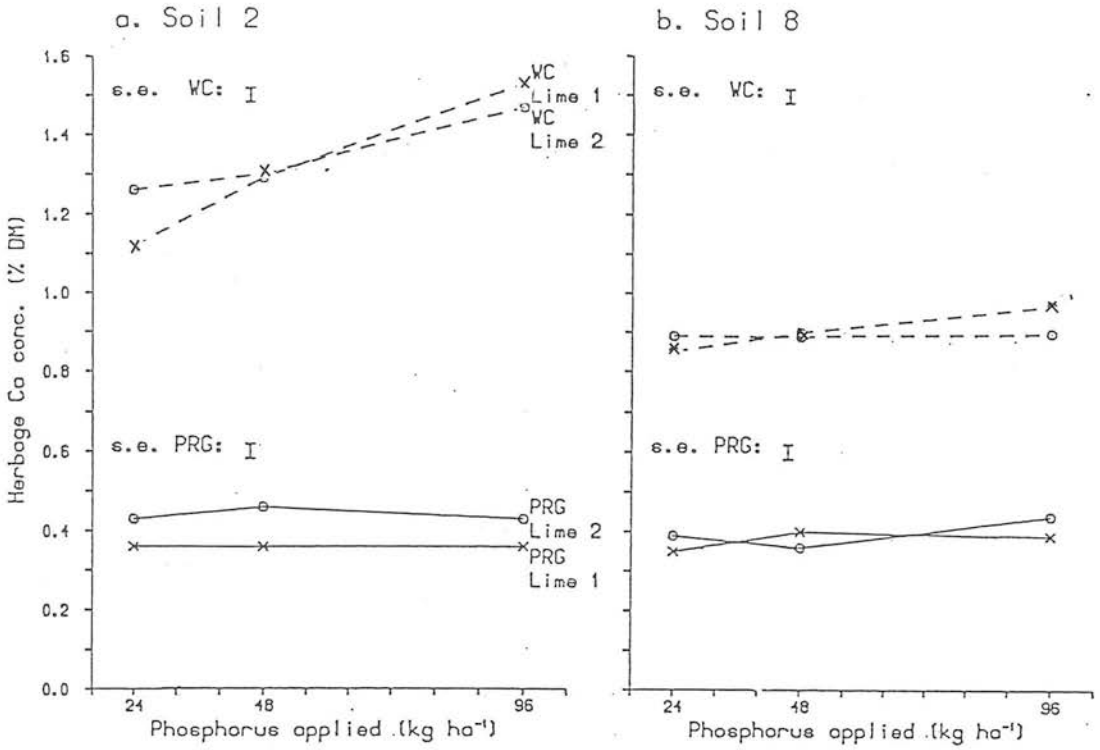

Figure 3.42: Herbage Ca concentration (% DM) for PRG and WC grown in soils 2 and 8 for 36 days at two lime levels and three P-levels.

Apart from the exceptions noted above the same basic trends were observed at the second as at the first harvest despite WC grown in soil 2 containing generally less Ca and PRG in soil 8 more Ca than at the second than at the first harvest.

Phosphorus

The P content of herbage grown in soil 8 was greater than that of herbage grown in soil 2 for all treatments (except WC at L1 and P3

where there was no difference). The soil difference was greater for PRG than for WC reflecting the higher P concentrations of PRG; the P content of PRG grown in soil 2 was greater than that of WC but to a lesser extent than in soil 8 (mean difference 28% compared to 60%).

Lime application had a significant effect ($p < 0.01$) on herbage P concentration but the effect was not consistent (Figure 3.43). The addition of P led to increased herbage P contents in all treatments, but there was no increase between P-levels 1 and 2 for PRG in soil 2 at lime level 2 or for WC in soil 8 between P-levels 2 and 3 at lime level 1.

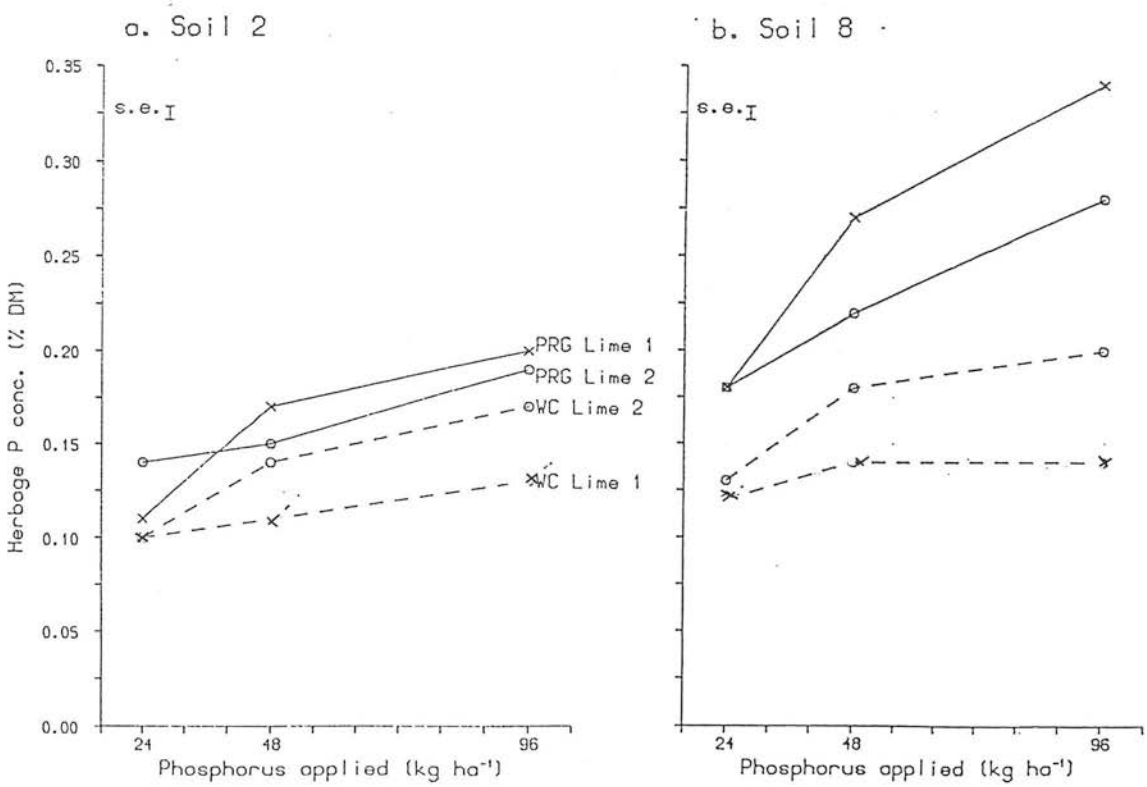

Figure 3.43: Herbage P concentration (% DM) for PRG and WC grown in soils 2 and 8 for 36 days at two lime levels and three P-levels.

The concentration of P in herbage had generally increased by the second harvest although for a few treatments, predominantly soil 2 at lime level 2, there was no change. The same basic pattern of results as at the first harvest was also observed at the second (Table A3.39).

2.5 Experiment 5: *An investigation into the effect of copper fertilizer on the content of copper, molybdenum and sulphur in white clover and perennial ryegrass grown in soils 2 and 8.*

The basic experimental design was:

2 soils (2, 8) x 2 species (WC, PRG) x 2 Cu levelx x 4 replicates with 2 sequential harvests.(Full details are given in Chapter II, Section 4.1.)

The data for herbage analyses are presented as histograms rather than tables, the numerical values can be found in Appendix 3.5. The two species grew at different rates, hence the first harvest of PRG was taken on day 30 (T1a) and that of WC on day 38 (T1b), both crops were harvested on the same day at the final harvest - day 50 (T2).

(a) Soil analysis

pH

The soil pH of all treatments decreased by approximately half a pH unit during the experiment (Table 3.7) (also observed in Experiments 2, 3 and 4). At the start of the experiment (T0) soil 2 had

Table 3.7: pH of soils 2 and 8 in which WC or PRG were grown with and without added Cu.

Soil	Copper addition (kg Cu ha^{-1})	*pH*					
		WC			PRG		
		†T0	T1b	T2	T0	T1a	T2
2	0	5.5	5.1	5.0	5.6	5.1	5.0
	10	5.5	5.1	5.0	5.4	5.0	5.0
8	0	5.3 ¨	5.1	5.0	5.5 ¨	5.1	5.0
	10	5.4	5.2	5.0	5.4	5.1	5.0

s.e.: T0 = 0.01, T1(a & b) = 0.04, T2 = 0.01

†T0, T1a, T1b, T2: 0, 30, 38 and 50 days, i.e. start of experiment, first (PRG, WC) and second harvests

slightly higher pHs than soil 8; and pots which were to have PRG growing in them had slightly higher pHs than pots which were to grow WC probably because of differences in sampling technique (see Chapter II, Section 4.2). There was no difference between the pHs of the pots in which PRG and WC had been grown at either harvest, in contrast to the results of Experiment 2A. The addition of Cu to the soils had no effect on pH at either harvest and only small and inconsistent effects at T0.

Extractable copper

Cu addition greatly increased the amount of extractable soil Cu, and the size of the increase approximately doubled during the course of the experiment. The increase in extractable soil Cu was particularly large for soil 8 (the soil which initially had the lowest soil Cu content) - twenty-fold at T0 (from 0.5 to 9.2 $mg\,kg^{-1}$ air-dry soil) and forty-fold at T2. In soil 2 the increases were three- and four-fold, respectively, at T0 and T2. The plant species growing in the soil did not affect extractable soil Cu concentrations (Table 3.8).

Table 3.8: Extractable soil Cu concentration of soils 2 and 8 in which WC or PRG were grown for 50 days with and without added Cu.

Soil	Copper addition (kg Cu ha^{-1})	*Extractable soil Cu concentration (mg kg^{-1} air-dry soil)*		
		†T0	T2	
			WC	PRG
2	0	3.8	4.3	4.3
	10	10.9	16.7	16.8
8	0	0.5	0.5	0.6
	10	9.2	18.9··	18.3··

s.e.: T0 - result of single analysis only, T2 = 0.48

†T0, T2: 0 and 50 days - start and conclusion of experiment.

Extractable molybdenum

Cu addition had no effect on the amount of extractable soil Mo in either soil at either sampling time. The amount of extractable Mo decreased between T0 and T2 by approximately a quarter to a third in soil 2 and by a half in soil 8 - the soil that initially contained the least Mo. The growth of PRG in the soils to which Cu had been added led to slightly lower final extractable soil Mo contents (Table 3.9).

Table 3.9: Extractable soil Mo concentration of soils 2 and 8 in which WC or PRG were grown for 50 days with and without added CU.

Soil	Copper addition (kg Cu ha^{-1})	*Extractable soil Mo concentration (mg kg^{-1} air-dry soil)*		
		†T0	T2	
			WC	PRG
2	0	0.085	0.058	0.054
	10	0.080	0.073	0.061
8	0	0.063	0.035	0.027··
	10	0.064	0.034	0.024

s.e.: T0 = 0.0109, T2 = 0.0049

†T0, T2: 0 and 50 days, i.e. start and conclusion of experiment.

Extractable sulphate

Cu addition had no effect on the amount of extractable SO_4^{2-}-S in either soil at either sampling time (as also observed for Mo). The content of SO_4^{2-}-S was a lot lower in soil 8 than soil 2 and the size of the difference approximately doubled between T0 and T2 from 25-35% to 50-70%, due to decreased SO_4^{2-}-S contents for soil 8, whilst those for soil 2 remained unaltered. The SO_4^{2-}-S contents of soils in which

PRG had been grown was approximately half that of soil in which WC had been grown for both soil 2 and soil 8 (Table 3.10).

Table 3.10: Extractable soil SO_4^{2-}-S concentration of soils 2 and 8 in which WC or PRG were grown for 50 days with and without added Cu.

Soil	Copper addition (kg Cu ha^{-1})	*Extractable soil SO_4^{2-}-S concentration (mg kg^{-1} air-dry soil)*		
		†T0	T2	
			WC	PRG
2	0	51	48	26
	10	54	51··	28
8	0	33	25··	11
	10	40	24	9

s.e.: T0 = 3.3, T2 = 1.0

†T0, T2: 0 and 50 days, i.e. start and conclusion of experiment.

(b) Herbage analysis

Dry matter production

Cu addition had no effect on herbage DM production except for a very small increase for WC grown in soil 8 at the first harvest ($p < 0.05$) (Figure 3.45a). There were large differences in production between soils and species - soil 8 produced 16-55% more herbage than soil 2, and PRG 49-68% more herbage than WC. The amount of DM production was greater at the second than the first harvest for PRG in soils 2 and 8 and for WC in soil 2, but there was no difference between the harvests for WC in soil 8 (Figure 3.44 and Table A3.40).

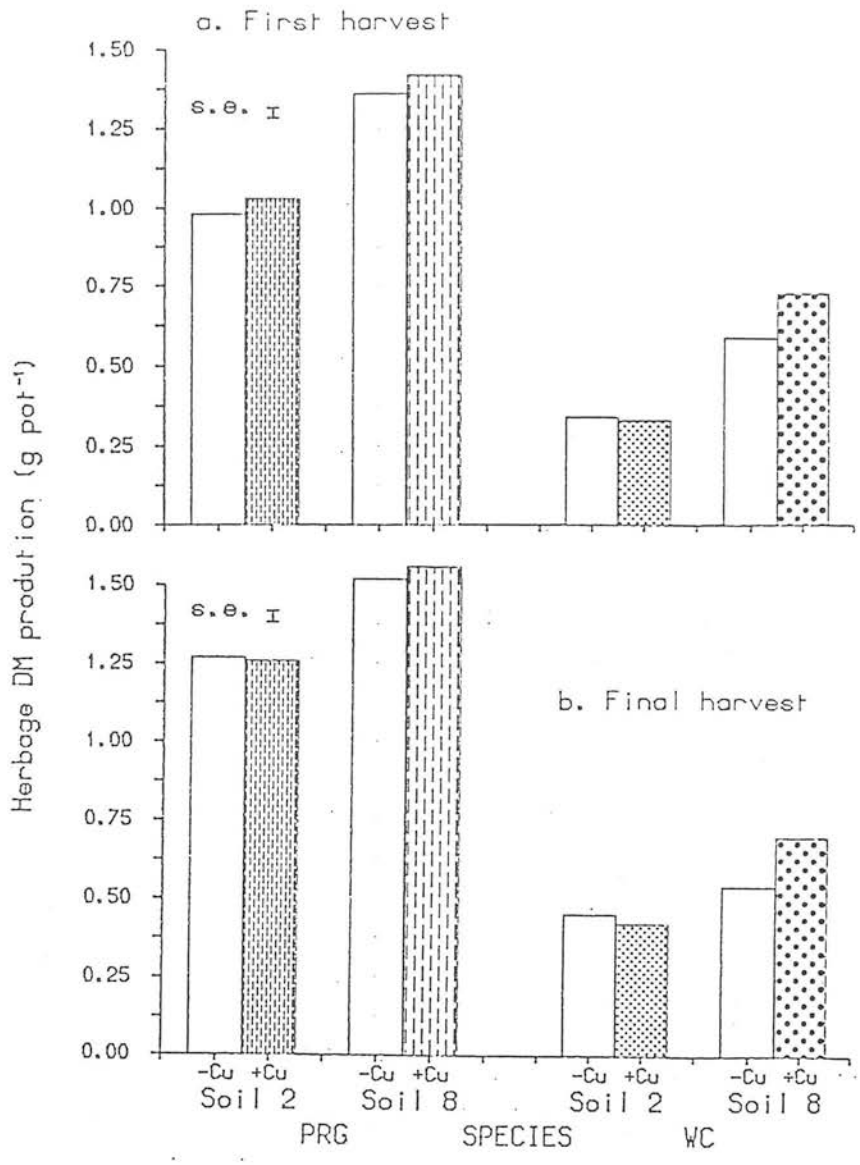

Figure 3.44: Herbage DM production (g pot^{-1}) of PRG and WC grown on soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

The absence of a response in plant dry matter production in soil 2 was predicted since soil 2 is considered to be Cu sufficient. It was expected, however, that a response might have been observed in soil 8. The EDTA-extractable soil Cu content of unameliorated soil 8 is 0.6 mg kg^{-1} oven-dry soil; which is both below the limit of 1.2 mg kg^{-1} given by Mackenzie (1974) for soils which can give "loss of

yield of cereals or ill health in sheep and cattle" (however, the applicability of this limit to grass Cu requirements is not known), and the SAC/SARI threshold for Cu deficient soils of 0.8 mg kg^{-1} air-dry soil (for mineral soils at pH 6.0).

Copper

Cu addition increased herbage Cu concentration for WC and PRG grown in soil 8 and for PRG grown in soil 2, but not for WC grown in soil 2; the relative increase was much greater in soil 8 (3.5- to 6-fold) than in soil 2 (1.5-fold) (Figure 3.45 and Table A3.41).

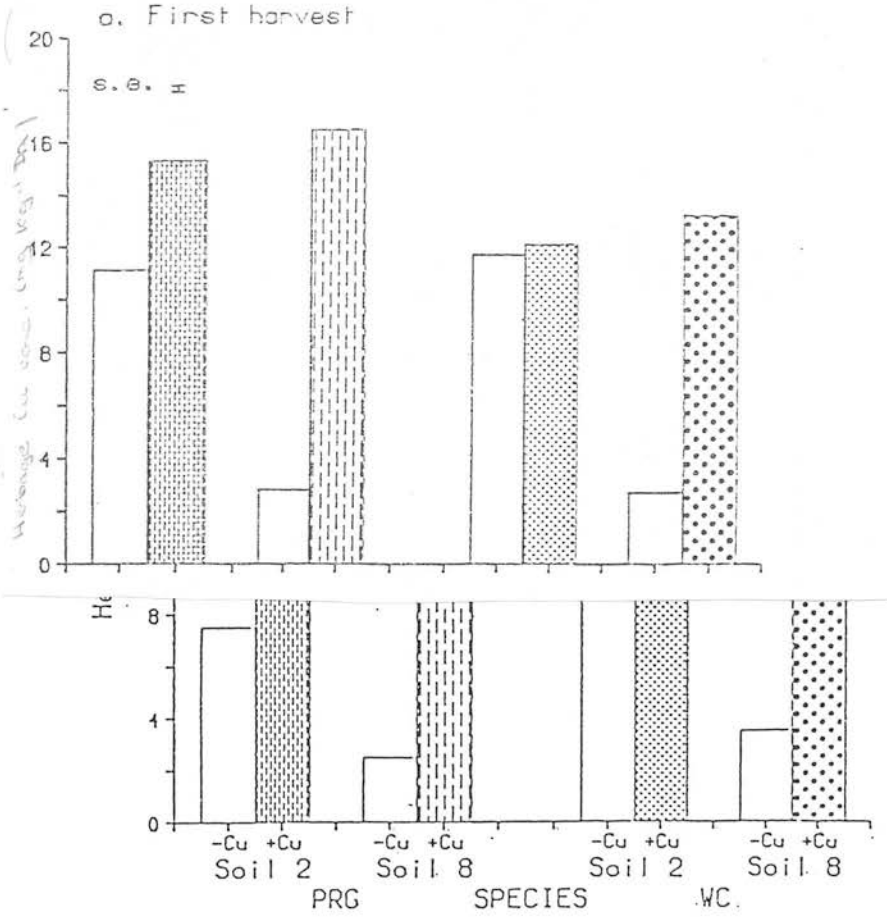

Figure 3.45: Herbage Cu concentration (mg kg^{-1} DM) of PRG and WC grown on soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

yield of cereals or ill health in sheep and cattle" (however, the applicability of this limit to grass Cu requirements is not known), and the SAC/SARI threshold for Cu deficient soils of 0.8 mg kg^{-1} air-dry soil (for mineral soils at pH 6.0).

Copper

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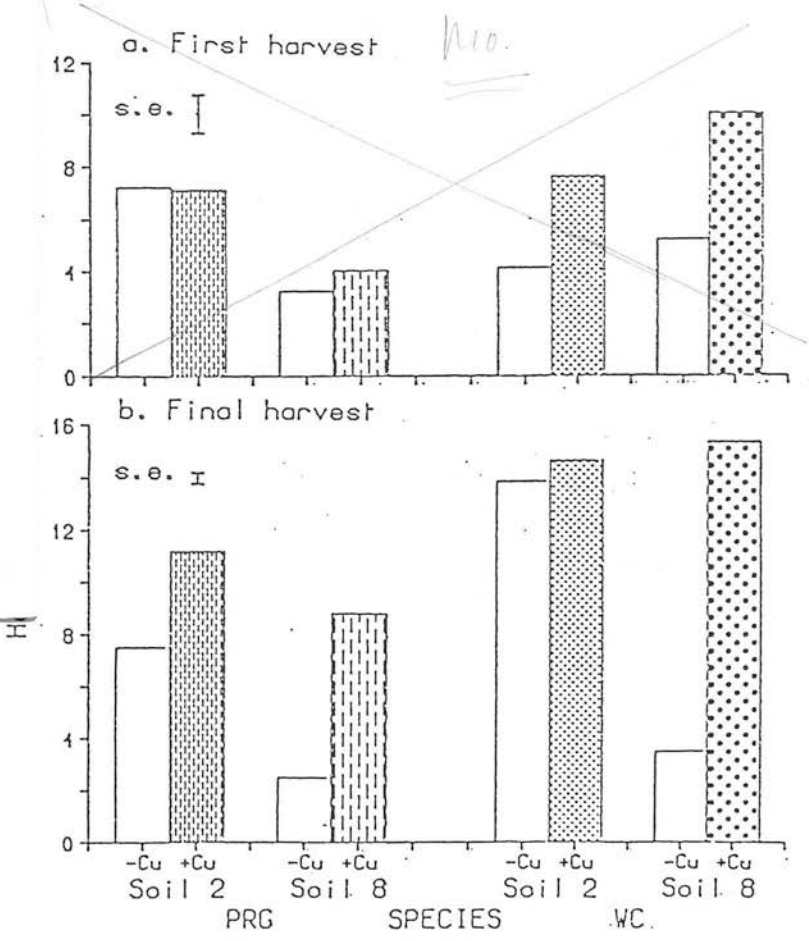

Figure 3.45: Herbage Cu concentration (mg kg^{-1} DM) of PRG and WC grown on soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

WC contained more Cu than PRG for all treatments at the second harvest and also for soil 2 with no added Cu at the first harvest. At the first harvest for soil 8 with no added Cu there was no difference between the Cu contents of PRG and WC, whereas for both soils with added Cu PRG contained more Cu than WC. The concentration of Cu increased between the harvests in WC but decreased in PRG.

Molybdenum

N.B. The interpretation of results at the second harvest (Figure 3.46b) is not straightforward because the values for the 4 replicates where Cu fertilizer was applied and WC grown in soil 8 were: 2.4, 4.5, 41.9 and 44.3. None of these values could be discarded as obvious outliers but because of their range equal variance does not apply to the analysis of variance. A standard error was computed by hand for the other seven treatments.

The addition of Cu increased the Mo concentration of WC but not PRG at the first harvest (Figure 3.46a) and apparently had no effect on either species at the second harvest (Figure 3.46b) with the exception noted above. There was no species difference in Mo concentration at the first harvest, although WC in soil 8 with added Cu appeared to contain more Mo than PRG, but at the second harvest PRG in soil 2 contained more Mo than WC, and no interpretation could be made for soil 8. The concentration of Mo in PRG increased between the harvests for both soils by *c.* 100% in soil 2 and *c.* 80% in soil 8, whilst WC concentration in soil 2 decreased by a third (Table A3.42).

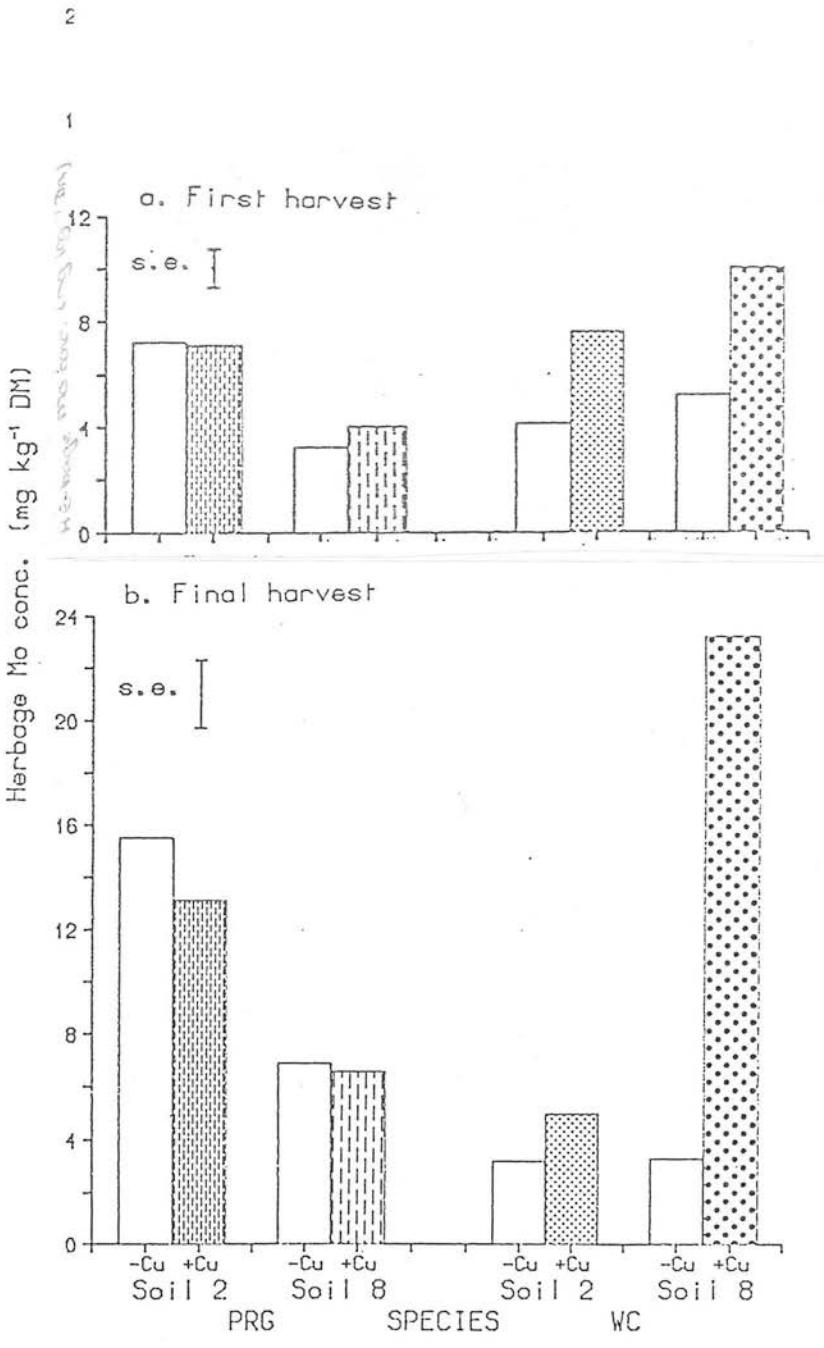

Figure 3.46: Herbage Mo concentration (mg kg^{-1} DM) of PRG and WC grown on soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

Sulphur

Cu addition had no effect on herbage S concentration at either harvest (Figure 3.47). Plants grown in soil 2 on average had a higher S content than plants grown on soil 8. At the first harvest for both soils PRG contained more S than WC, but at the second the concentrations in PRG and WC were similar in soil 2, whereas in soil 8 the concentration of S in WC was twice that of PRG. The concentration of S in PRG decreased between the two harvests (as in other experiments) and also for WC in soil 8, but remained unchanged for WC in soil 2.

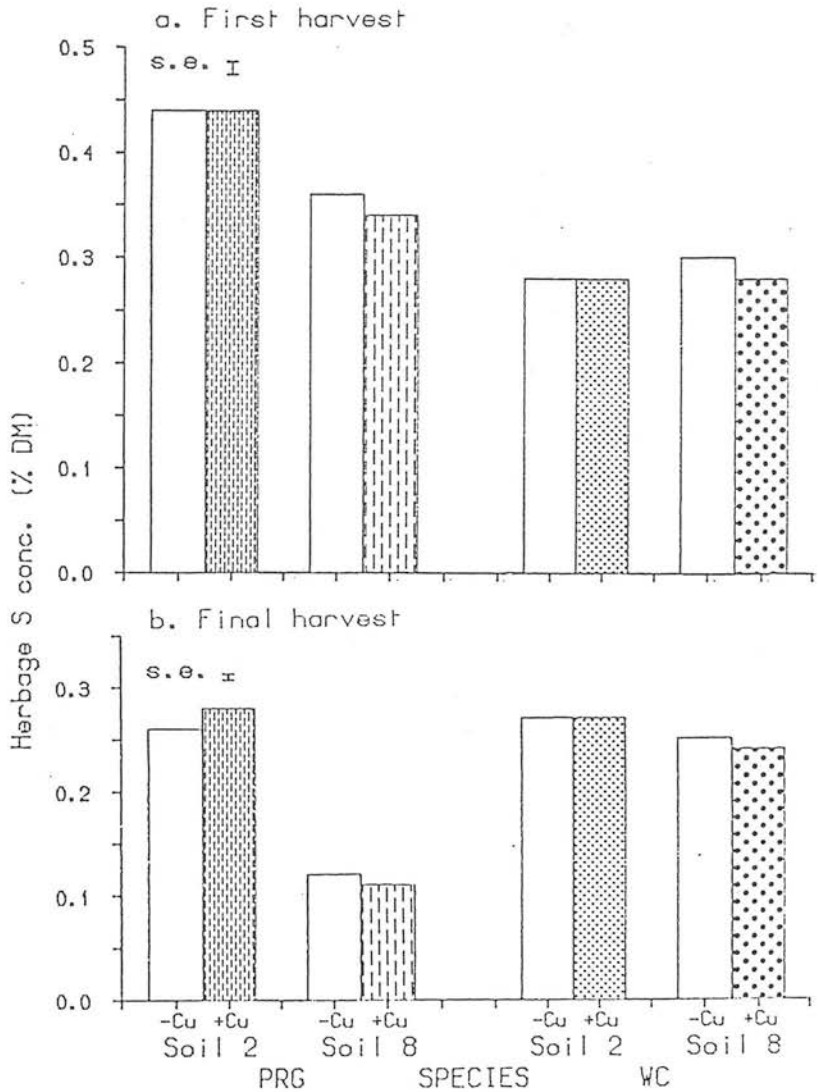

Figure 3.47: Herbage S concentration (% DM) of PRG and WC grown on soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

2.6A Experiment 6A: *An investigation into the effect of two different moisture regimes on the copper, molybdenum, sulphur and nitrogen content of soils 2 and 8 incubated for three months at two pHs.*

The basic experimental design was:

2 soils (2, 8) x 2 pH (unlimed and limed - lime levels 0 and 1) x 2 moisture regimes (moist, waterlogged - regimes 1 and 2) x 3 replicates, incubated at 20°C for three months and sampled on days 3, 10, 24, 53 and 94. (Full details are given in Chapter III, Section 4.1).

The data for all soil variables measured are presented as plots of the variable against time (numerical values are given in Appendix 3.6). The results of the chemical analyses are given on an oven-dry basis, since the analyses were performed on fresh soil; the results are therefore not directly comparable with the results of other experiments which are expressed on an air-dry basis.

(a) Soil analysis

Moisture percentage

There was more water held in soil 8 than soil 2, as soil 8 contained more OM and hence had a greater water holding capacity (Table 3.11). The moisture percentage of the waterlogged treatments apparently increased slightly during the course of the experiment but this was probably a reflection of the difficulties encountered in sampling these wet and fluid soils.

Table 3.11: % Moisture of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture regime[1]	Lime level[2]	% H_2O				
			Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	26	31	33	31	30
		1	29	31	30	30	31
	2	0	42	45	45	45	50
		1	44	44	44	44	50
8	1	0	44	46	44	43	45
		1	47	46	45	43	45
	2	0	58	59	61	62	65
		1	57	59	60	63	66

s.e.: Day 3 = 1.1, Day 10 = 1.0, Day 24 = 1.8, Day 53 = 1.3, Day 94 = 1.6

[1]1, 2: 60% FC, waterlogged

[2]0, 1: unlimed, limed to pH 6.0.

pH (fresh soil)

The pH of soil 8 was lower than the pH of soil 2 at all times in the unlimed soils under both moisture regimes and in the waterlogged limed soil, but was higher in the moist (60% FC) limed soil (Figure 3.48).

At the first two sampling times (days 3 and 10) moisture regime had no effect on soil pH but at 24, 53 and 94 days there was an effect, particularly in soil 2. The pH of waterlogged unlimed soil 2 rose steadily from 3.5 on day 3 to 5.3 on day 53 and then dropped slightly to 5.0, whereas the pH of the moist unlimed (control) soil remained constant at pH 3.7 throughout the incubation period. When soil 2 was limed and then incubated moist the pH initially dropped (5.9 to 5.5 between days 3 and 53) and then remained steady, whereas when it was incubated

waterlogged the pH initially increased - but to a much smaller extent than had been observed in the unlimed soil (6.0 to 6.3 between days 3 and 53), and then remained steady (Figure 3.48a). The changes in soil pH that occurred in soil 8 were very small in comparison to those in soil 2 (Figure 3.48b) which may be attributed to the higher buffering capacity of soil 8 (see also Table A3.44).

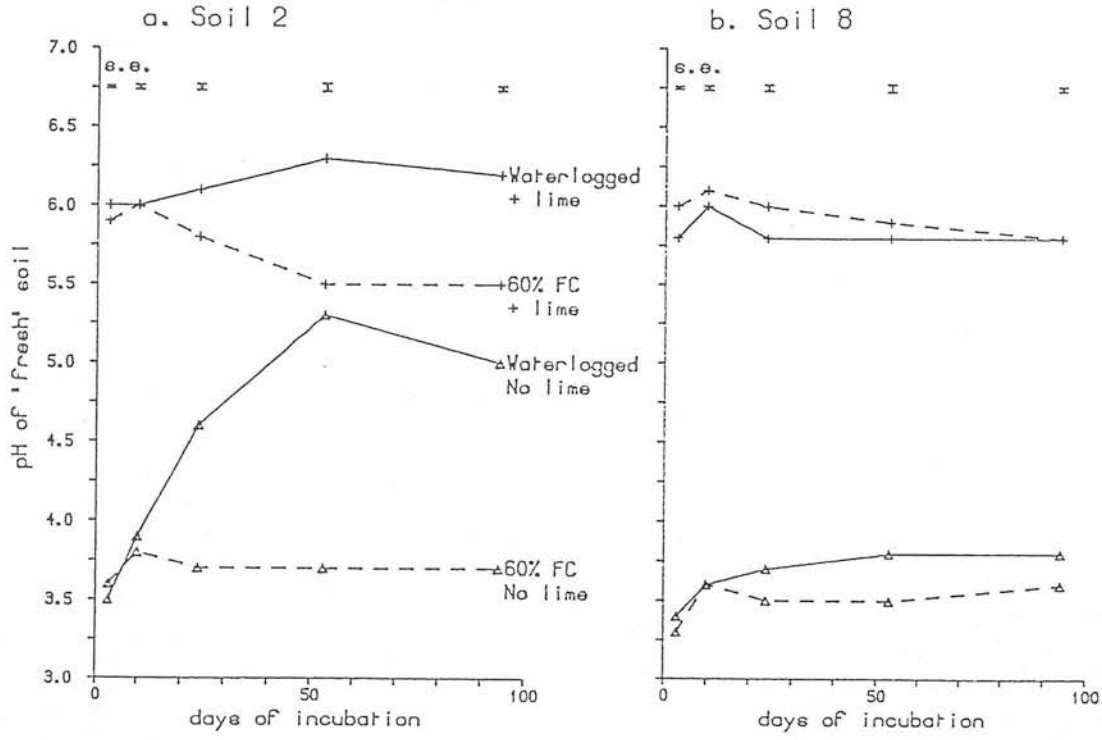

Figure 3.48: pH of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

The effect of waterlogging was greater in the unlimed than the limed soil because the latter was closer to the plateau of the pH response curve. Ponnamperuma (1972, 1984) states that pHs of waterlogged soils will stabilise at pH 6.7 to 7.2 within a few weeks of waterlogging, however this was not observed here. A possible reason why the pHs

did not stabilise near neutrality could be because of the relatively high OM and low reducible Fe contents of the soil producing an acidifying effect in the soils (Table 3.2).

Extractable copper

Soil 2 had greater extractable soil Cu contents than soil 8 (as in all the experiments), the ranges were 1.0 to 2.9, and 0.16 to 0.36 mg Cu kg^{-1} oven-dry soil respectively; so the two soils were separated for the statistical analysis (Table A3.45).

Although the addition of lime did affect the extractable Cu content of soil 2 ($p < 0.05$ on days 3 and 53, $p < 0.01$ on day 10, $p < 0.001$ on day 24 and non-significant on day 94) the effect of changing the moisture regime was greater ($p < 0.001$ at all sampling times). The Cu content of the moist unlimed (control) soil was unaffected by incubation and remained at 2.2 - 2.3 mg kg^{-1} oven-dry soil, but that of the moist limed soil after an initial small increase (2.1 to 2.3 mg kg^{-1} oven-dry soil between days 3 and 10) decreased slightly to 1.9 mg kg^{-1} by day 53 and then remained constant. In contrast, in both of the waterlogged treatments the soil Cu concentrations dropped by approximately a half between days 3 and 24, then increased slightly between days 24 and 53 (greater in the limed soil) and then decreased again between days 53 and 94 (greater in the unlimed soil) (Figure 3.49a).

In soil 8 there was little effect either of liming or of soil water content on the concentrations of extractable soil Cu (Figure 3.49b).

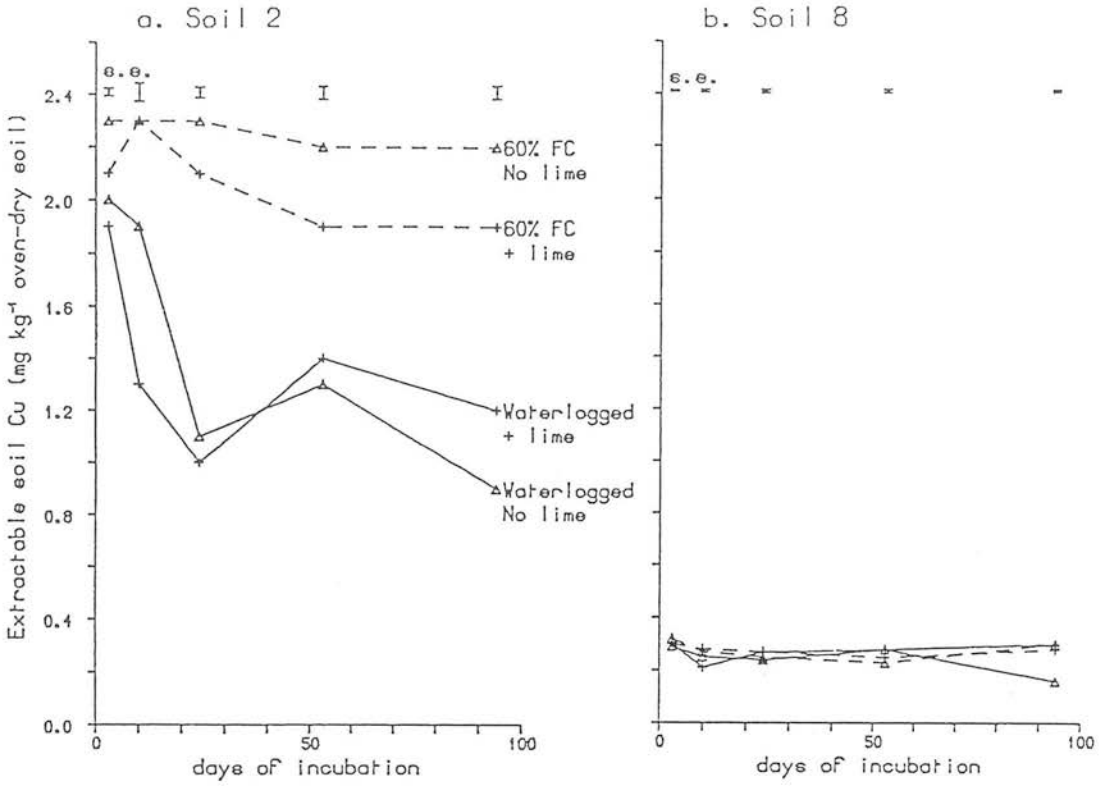

Figure 3.49: Extractable Cu of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Extractable molybdenum

The two treatments applied (lime addition and varying soil water contents) had a greater effect on the extractable soil Mo concentration of soil 2, than that of soil 8 although initially (day 3) they both contained similar Mo concentrations (Table A3.46).

The Mo concentration in the moist unlimed (control) treatment of soil 2 changed little during the incubation, but those of the other three treatments decreased by 2½ to 6 times (Figure 3.50a). Liming increased the extractable Mo content of soil 2 and the difference became greater as the length of incubation increased. The significance of the effect of waterlogging increased as the incubation progressed (non-

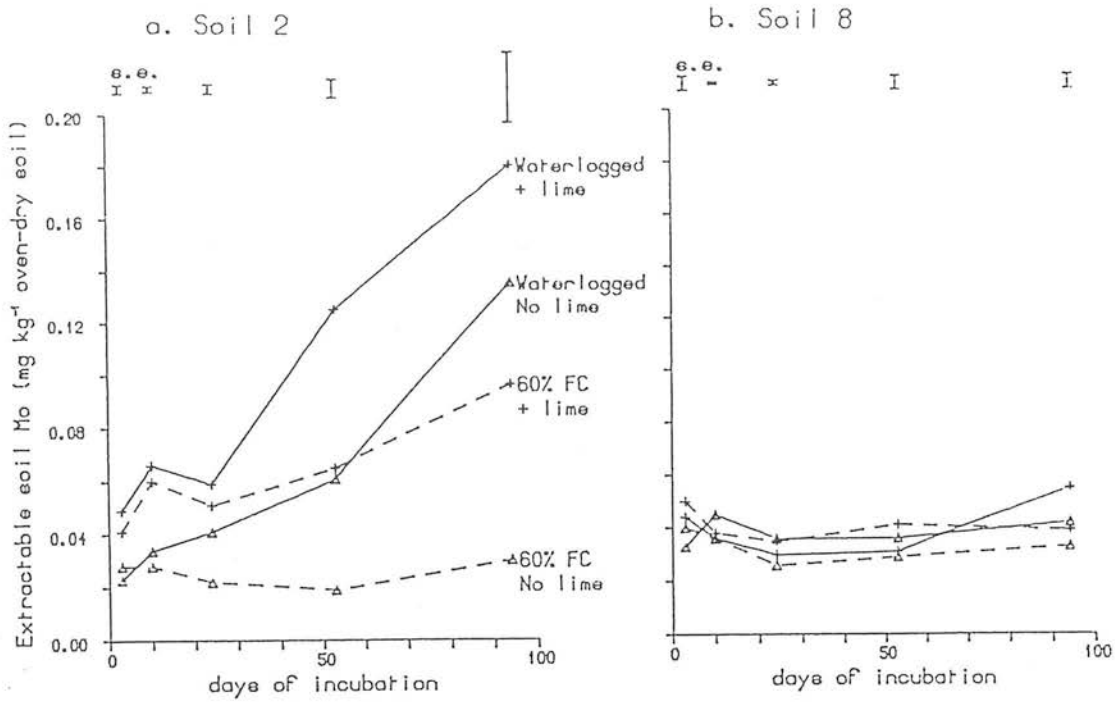

Figure 3.50: Extractable Mo of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

significant on day 3, $p < 0.05$ on day 10, $p < 0.01$ on day 24, $p < 0.001$ on days 53 and 94) and waterlogged treatments always contained more Mo than moist treatments (except on day 3) - 20-350% more depending on the treatment and sampling time.

The extractable soil Mo concentration of soil 8 was little affected by either lime addition or changing soil water contents (Figure 3.50b).

Extractable sulphate-sulphur

The amounts of extractable soil SO_4^{2-}-S in the moist unlimed (control) treatments of soil 2 and soil 8 were similar but there were differences in the other three treatments (Table A3.47).

Although lime addition did have an effect on soil SO_4^{2-}-S contents the effect of waterlogging was greater, obscuring the effect of lime in

the waterlogged soils. Lime addition increased the amount of soil extractable SO_4^{2-}-S (also observed in Experiment 1) by about 100% in both soils. Waterlogging led to a decline in SO_4^{2-}-S concentrations which occurred particularly rapidly in the limed treatments and was greater in soil 2 than soil 8. Both waterlogged treatments in soil 2 had an initial increase in SO_4^{2-}-S concentrations (84% in the limed and 28% in the unlimed) but a similar increase was not observed in soil 8. Also whereas in soil 2 the SO_4^{2-}-S contents reached the lower limit of detection, in soil 8 they stabilised at *c.* 10 mg kg^{-1} oven-dry soil (Figure 3.51).

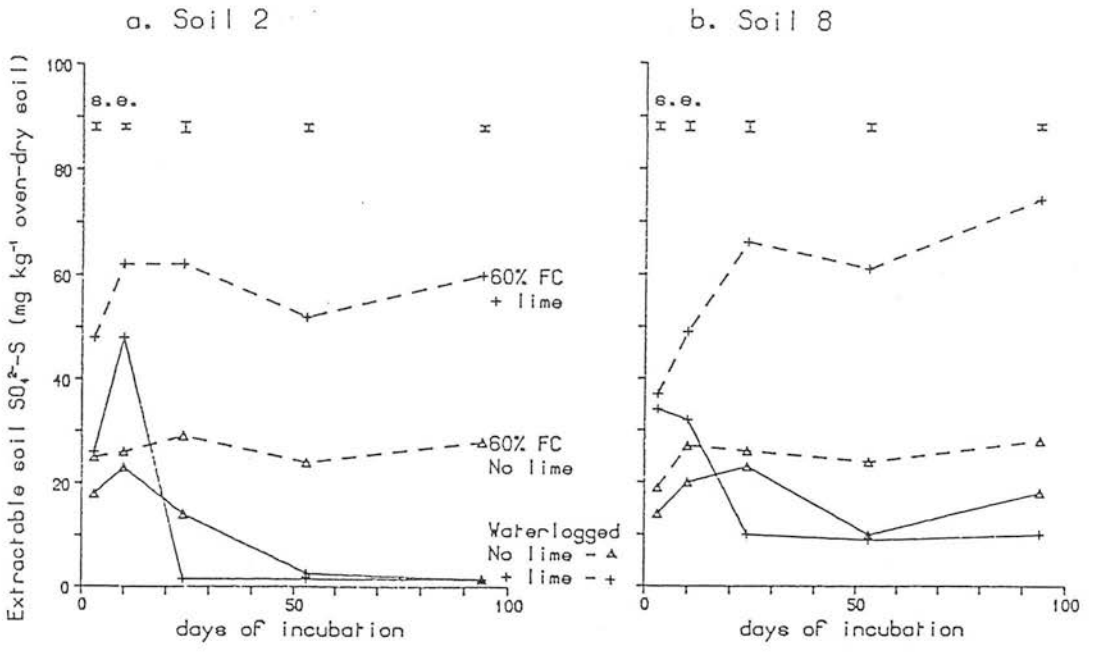

Figure 3.51: Extractable SO_4^{2-}-S of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

As the incubation progressed H_2S became detectable (was smelt) in the waterlogged treatments. The gas was first noticeable from soil 2, this is consistent with the speed at which the concentration of SO_4^{2-}-S that could be detected decreased.

Soil nitrogen - extractable ammonium and nitrate

The extractable soil NH_4^+-N and NO_3^--N concentrations were monitored in order to observe the development of reducing conditions (Figures 3.52 and 3.53).

Ammonium:

The concentrations of extractable soil NH_4^+-N were greater in soil 8 than soil 2, and the magnitude of the differences increased as the length of incubation increased (Table A3.48).

The addition of lime generally increased the concentrations of NH_4^+, particularly for soil 8 (except soil 2 moist on days 53 and 94) because the process of mineralization was favoured. The effect of waterlogging was small - towards the end of the incubation of soil 2 waterlogging increased soil NH_4^+-N concentrations, and for soil 8 waterlogged treatments always contained less NH_4^+-N than moist treatments (Figure 3.52).

Nitrate:

The concentrations of extractable soil NO_3^--N were similar in soil 2 and soil 8, although at the first two sampling times the concentration was higher in limed soil 8 than limed soil 2 (Table A3.49).

The concentrations of NO_3^--N were generally at the lower limits of detection in the unlimed soils but were detectable in the limed soils where mineralization had been encouraged. Hence the different soil water contents had no effect on the NO_3^--N content of unlimed soils nor did they affect the NO_3^--N contents of the limed soils until days 53 and 94 when the NO_3^--N concentrations of the moist soils increased five-fold while those of the waterlogged soils remained little changed (Figure 3.53).

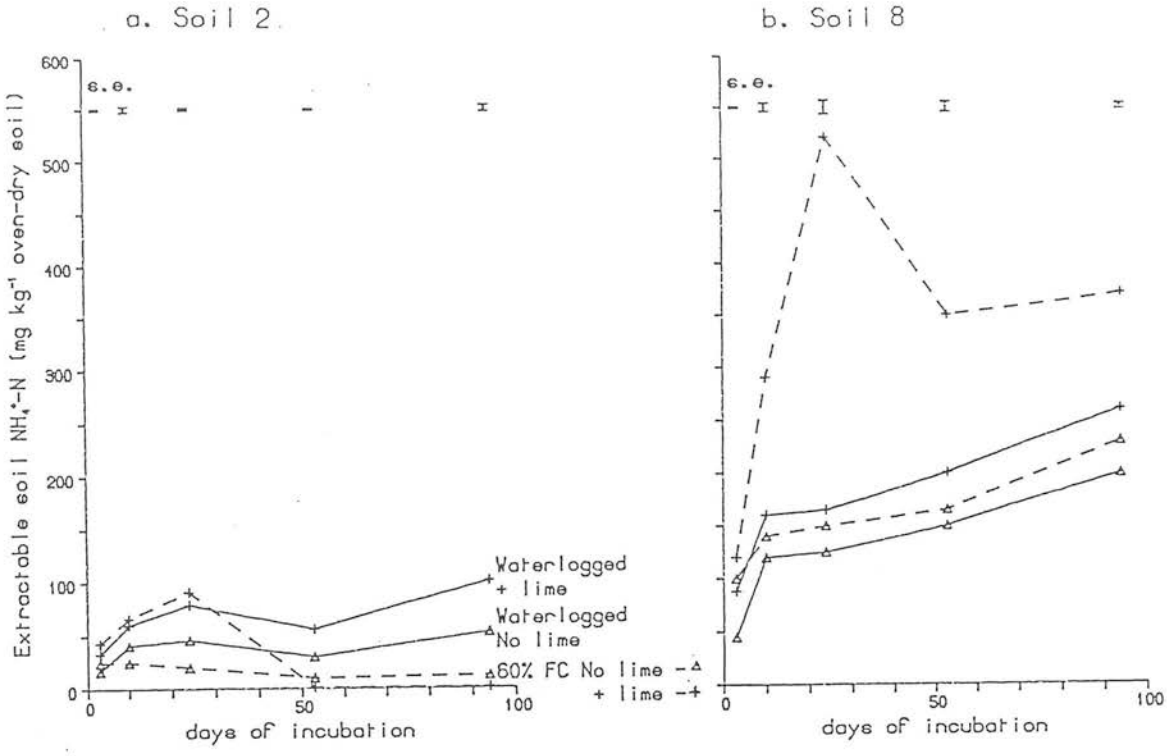

Figure 3.52: Extractable NH_4^+-N of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

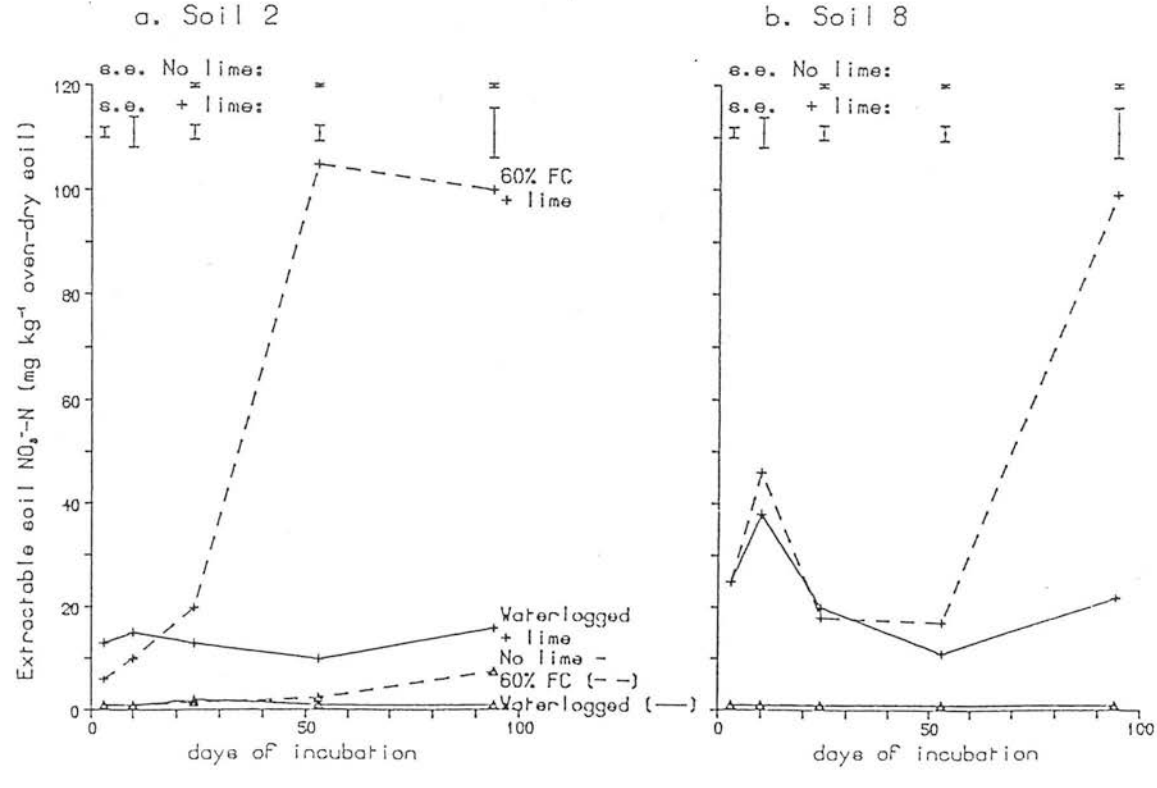

Figure 3.53: Extractable NO_3^--N of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

When a soil is waterlogged anaerobic conditions are present and hence mineralization cannot continue beyond the production of NH_3; therefore, the NH_3 content of the soil increases overall since the rate of immobilization of the NH_4^+ ions by chemical fixation (absorption by clays and colloids) and biological immobilization, and volatilization of NH_3 does not exceed the rate of NH_4^+ production by mineralization. Since NO_3^- is not formed in waterlogged soils the concentration present remains unaltered and may even decrease slightly due to denitrification (Ponnamperuma, 1984).

2.6B Experiment 6B: *An investigation into the effect of two watering regimes on the copper, molybdenum and sulphur content of perennial ryegrass grown in soils 2 and 8 which had previously been subjected to different moisture regimes.*

The basic experimental design was:

2 soils (2, 8) x 3 pre-treatments (dry, moist, waterlogged) x 2 watering regimes (*c.* 60% and 100% FC) x 3 replicates; limed and growing PRG for 36 days (full details are given in Chapter II, Section 4.1).

The experiment was initially designed to look at WC also, but the growth of this species was so poor (see herbage DM production section) that insufficient herbage was obtained for chemical analyses, therefore only PRG results are given. The results of the soil analyses are presented as tables and those of the plant analyses as histograms; the numerical data is presented in Appendix 3.6.

(a) Soil analysis

Moisture percentage

The greater water holding capacity of soil 8 was reflected in the higher moisture percentages of soil 8 compared to soil 2 (Table 3.12).

Table 3.12: % Moisture of soils 2 and 8 in which PRG was grown for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	% H_2O					
		[1]Dry		Moist		Waterlogged	
		[2]T0	T1	T0	T1	T0	T1
2	60	25	25	25	24	26	24
	100	38	37	35	34	33	44
8	60	41	38	42	40	46	48
	100	54	54	50	53	52	51

s.e.: T0 = 1.0, T1 = 1.1

[1]Soil pre-treatment

[2]T0, T1: days 0 and 36 - start and conclusion of experiment.

pH (fresh soil)

Although there were significant differences in the pHs of different treatments and treatment combinations the real differences were small and not of primary importance with regard to the effects of the treatments on soil and plant Cu, Mo and S concentrations (Table 3.13).

The most noticeable differences were the higher pHs at the start and conclusion of the pre-treatment dry soil compared to the pre-treatment moist and pre-treatment waterlogged. This difference arose because the lime application to the pre-treatment dry soil was made two weeks

before the start of the experiment rather than four months as for the other two pre-treatments. Another noteworthy difference was the marginally higher pH at the end of the experiment for the soils which had been maintained at *c.* 100% FC rather than 60% FC.

Table 3.13: pH (fresh) of soils 2 and 8 in which PRG was grown for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	*Soil pH (fresh)*					
		[1]Dry		Moist		Waterlogged	
		[2]T0	T1	T0	T1	T0	T1
2	60	6.0	5.7	5.4	5.5	5.5	5.4
	100	6.0	5.8	5.4	5.7	5.5	5.6
8	60	6.2	5.7	5.3	5.3	5.8	5.5
	100	6.0	5.7	5.3	5.6	5.8	5.6

s.e.: T0 = 0.07, T1 = 0.03

[1]Soil pre-treatment

[2]T0, T1: days 0 and 36 - start and conclusion of experiment.

The pH of the moist incubated soil 2 had not altered from that at the end of Part A of the experiment, but the pH of soil 8 had decreased by 0.5 unit although it had remained relatively stable throughout most of the incubation (Figure 3.48). For the waterlogged soils the situation was reversed; whereas the pH of soil 2 was reduced (by 0.7 unit) on reoxidation (using up H^+ ions) that of soil 8 was unaltered.

Extractable copper

The extractable soil Cu concentration was lower in soil 8 than in soil 2. There was no difference in soil Cu concentrations between the

two watering regimes in either soil for any pre-treatment, but there was an effect of pre-treatment - waterlogged > moist ≃ dry. The effect of pre-treatment was larger in soil 2 than soil 8 (dry/moist to waterlogged 300% difference in soil 2 *c.f.* 60% in soil 8); but was smaller at the end of the experiment than the start for both soils due to decreased Cu contents of the waterlogged soils (Table 3.14).

Table 3.14: Extractable Cu of soils 2 and 8 in which PRG was grown for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	*Extractable soil Cu (mg kg^{-1} oven-dry soil)*					
		†Dry		Moist		Waterlogged	
		[2]T0	T1	T0	T1	T0	T1
2	60	2.1	1.8	2.0	1.8	6.8	5.2
	100	1.9	1.8	1.9··	1.8	6.6	5.3
8	60	0.25	0.21	0.28	0.25	0.46	0.32
	100	0.24	0.25	0.27	0.24	0.45··	0.34

s.e.: Soil 2 T0 = 0.19 T1 = 0.08
Soil 8 T0 = 0.025 T1 = 0.017

†Soil pre-treatment

[2]T0, T1: days 0 and 36 - start and conclusion of experiment.

Extractable molybdenum

The extractable soil Mo concentration was lower in soil 8 than in soil 2 (except for pre-treatment dry at the start of the experiment); and the size of the difference was larger at the end of experiment, because whereas the Mo content of soil 2 increased that of soil 8 decreased (Table 3.15).

Table 3.15: Extractable Mo of soils 2 and 8 in which PRG was grown for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	*Extractable soil Mo (mg kg^{-1} oven-dry soil)*					
		†Dry		Moist		Waterlogged	
		[2]T1	T0	T1	T0	T1	T0
2	60	0.046	0.060	0.053	0.055	0.066	0.145
	100	0.056	0.064	0.051	0.076	0.067	0.125
8	60	0.063	0.044	0.029	0.022··	0.042··	0.022
	100	0.054	0.037	0.035	0.019	0.031	0.025

s.e.: T0 = 0.0039, T1 = 0.0043

†Soil pre-treatment

[2]T0, T1 = days 0 and 36 - start and conclusion of experiment.

Watering regime had no effect on soil Mo content but the pre-treatment that had been applied did; generally for soil 2 the order was waterlogged > moist ≃ dry and for soil 8 the order was dry > moist ≃ waterlogged.

Extractable sulphate-sulphur

There was no difference between the extractable soil $SO_4^{2-}S$ concentrations of soils 2 and 8 at the start of the experiment, but at the conclusion soil 8 contained less than soil 2 (except pre-treatment moist maintained at 60% FC) (Table 3.16).

By the end of the experiment the $SO_4^{2-}S$ content was more in the soils which had been maintained at 60% FC than those which had been maintained at 100% FC - by 40 to 90% (there was no difference for soils pre-treatment waterlogged). The effect of pre-treatment was moist > dry > waterlogged with regard to $SO_4^{2-}S$ concentrations for both soils.

There was a general decline in the concentration of extractable soil SO_4^{2-}-S between the start and conclusion of the experiment.

Table 3.16: Extractable SO_4^{2-}-S of soils 2 and 8 in which PRG was grown for 36 days at 60°C or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	*Extractable soil SO_4^{2-}-S (mg kg^{-1} oven-dry soil)*					
		†Dry		Moist		Waterlogged	
		[2]T0	T1	T0	T1	T0	T1
2	60	50	40	59	53	37	28
	100	51	25	60	28	38	17 ··
8	60	42	17	78	48	28	13
	100	44	9	68	30	27 ··	11

s.e.: T0 = 2.8, T1 = 2.0

†Soil pre-treatment

[2]T0, T1 = days 0 and 36 - start and conclusion of experiment.

(b) Herbage analysis

Dry matter production

There were very large differences in the amounts of herbage produced by WC and PRG. WC produced much less herbage than PRG, despite being harvested one week later, and generally did not produce sufficient herbage for chemical analysis (Table 3.17). DM production was better for both species in soil 8 rather than in soil 2, and in pots kept at 100% FC rather than at 60% FC where water supply had obviously been a limitation to production.

There was more herbage produced in the pre-treatment dry pots than in either of the other two pre-treatments. This was probably because although all pots had basal NPK fertilizer added at the start of

Table 3.17: Herbage DM production of PRG and WC grown in soils 2 and 8 for 36 and 43 days respectively at 60% or 100% FC after the soils had been stored dry, moist or waterloggd for three months.

Soil	Watering regime (% FC)	*Herbage DM production (g pot^{-1})*					
		PRG			WC		
		†Dry	Moist	Water-logged	Dry	Moist	Water-logged
2	60	1.16	0.65	0.29	0.18	0.05	0.02
	100	2.93	2.86	1.68	1.24	0.54	0.08
8	60	2.22	1.11	1.22	0.44	0.19	0.09
	100	4.27	3.18	3.15	2.11	0.52	1.11

s.e.: PRG = 0.103, WC = 0.073

†Soil pre-treatment.

Experiment 6B the availability of other nutrients had been affected by the moist/waterlogged incubation period. There would also have been an initial flush of mineralization and nutrient release when the dry soil was rewetted for plant growth (the 'Birch' effect), this flush would have occurred at the start of the incubation period for the other two pre-treatments. For PRG grown in soil 8 similar amounts of herbage were produced from the moist and waterlogged pretreated pots but for soil 2 moist > waterlogged.

Copper

The concentration of herbage Cu was 3 to 6 times greater in PRG grown in soil 2 rather than in soil 8 (Table 3.50). Watering regime did not affect the Cu content of PRG grown in soil 8 but for soil 2 plants grown at 100% FC contained less Cu than plants grown at 60% FC (Figure 3.54). In both soils plants grown in the pre-treatment waterlogged

soil contained more Cu than plants grown in the dry or moist pre-treatment soils. There was an apparent interaction of pre-treatment and watering regime in soil 2, pots at 60% FC waterlogged > dry > moist but for pots at 100% FC waterlogged > moist > dry. The order in soil 8 was waterlogged > dry > moist.

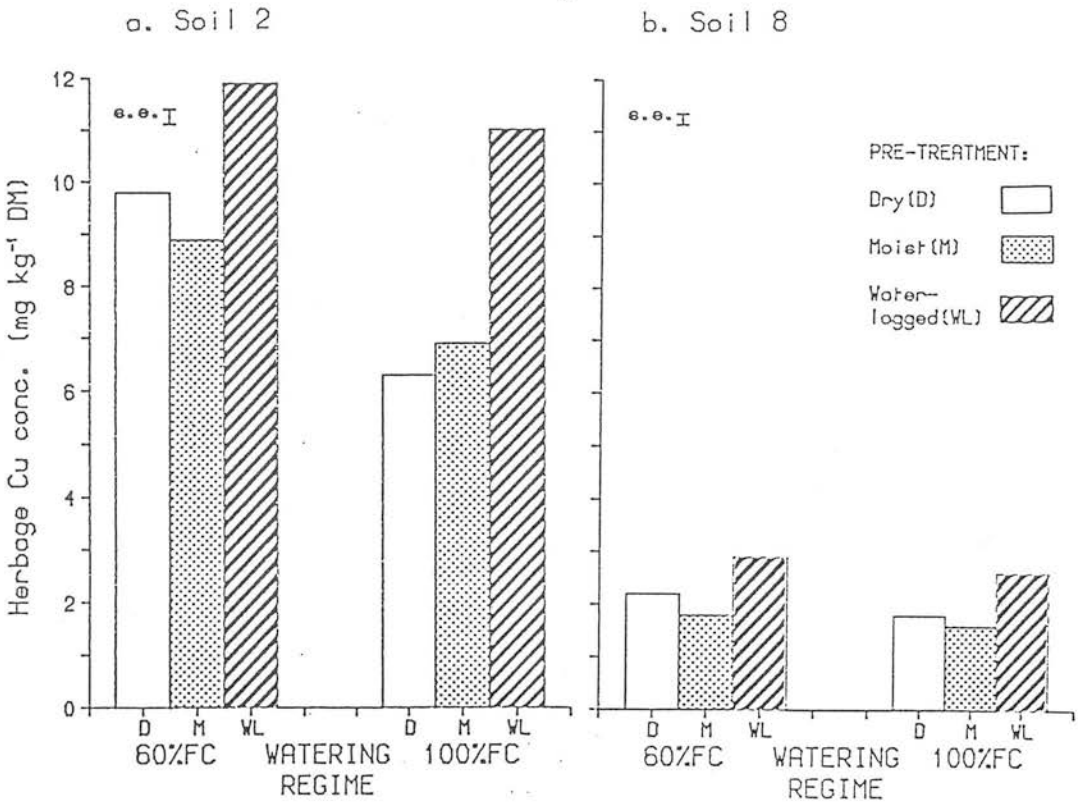

Figure 3.54: Herbage Cu (mg kg^{-1} DM) concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Molybdenum

The herbage Mo concentration of PRG grown in soil 2 was greater than that of PRG grown in soil 8 (by 70 to 300%) (Table A3.51). Watering regime had no effect on the Mo content of PRG grown in soil 2 but for plants grown in soil 8 those maintained at 100% FC contained less Mo than those maintained at 60% FC (Figure 3.55).

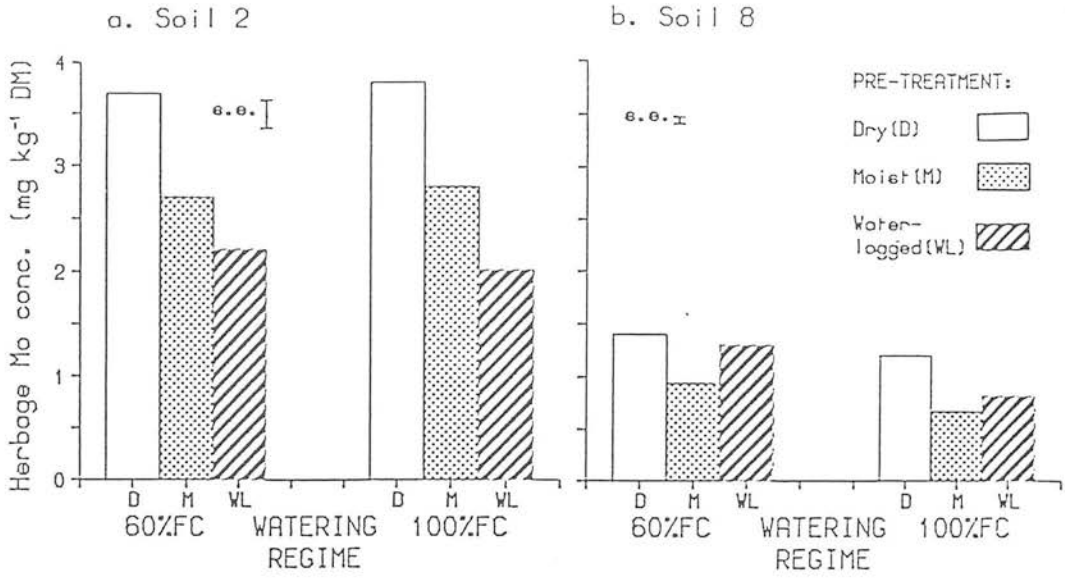

Figure 3.55: Herbage Mo (mg kg^{-1} DM) concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

The effect of pre-treatment was different in the two soils and for soil 8 there seemed to be an interaction with watering regime. In soil 2 the order was: dry > moist > waterlogged; and for soil 8: pots at 60% FC dry ≃ waterlogged > moist, and pots at 100% FC dry > waterlogged > moist.

Sulphur

The herbage S concentration was greater for plants grown in soil 2 than soil 8 for the dry and waterlogged pre-treatments but less for the moist pre-treated soil maintained at 100% FC and no different for this pre-treatment maintained at 60% FC (Table A3.52). Plants grown at 100% FC contained less S than plants grown at 60% FC for both soils and all pre-treatments (Figure 3.56).

The effect that soil pre-treatment had on plant S content varied with the two soils: for soil 2 the order was dry = waterlogged > moist and for soil 8 moist > dry > waterlogged.

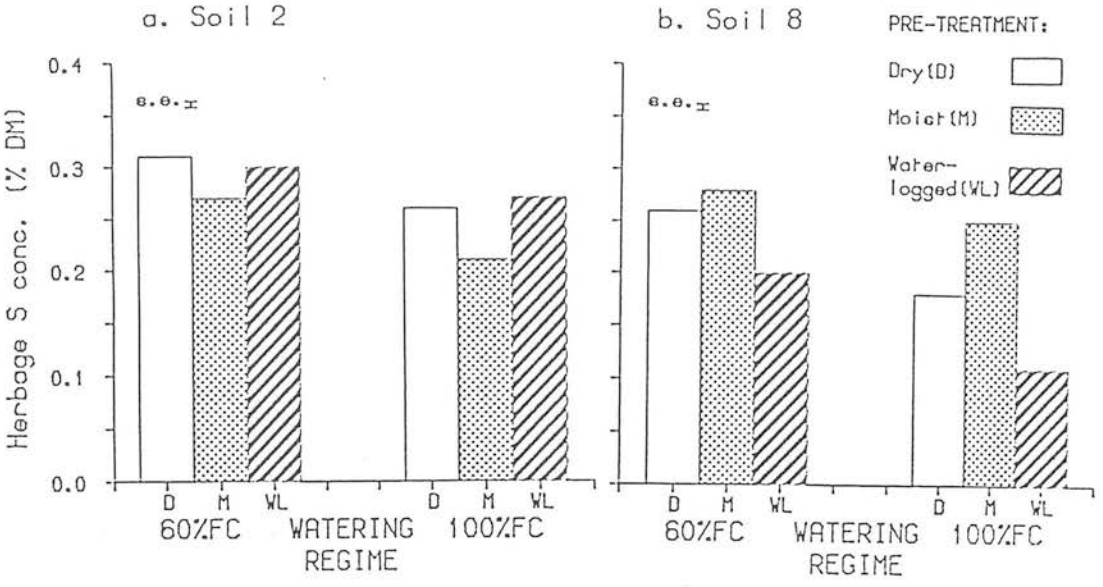

Figure 3.56: Herbage S (% DM) concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

CHAPTER IV

Discussion

The following chapter will consider the main observations from the experimental work presented in this thesis. The discussion will be constructed in a similar manner to the Literature Review (Chapter I) considering, in turn, the various factors that influence, or are affected by hill land improvement. The section for each factor will consider, for each variable under consideration (generally Cu, Mo and S concentrations): first the soil aspects and then the plant aspects and soil x plant relationships. The penultimate section of the chapter will consider how the various factors studied have affected the availability of herbage Cu to grazing sheep - relating herbage Cu, Mo and S concentrations; finally a brief summary discussion will be presented incorporating suggestions for future work.

1. SOIL

In the majority of the experiments presented in this thesis only soils 2 (Glensaugh, Birnie) and 8 (Sourhope, Peaty Podzol) were used, although in Experiment 2 soil 4 (Hartwood) was also used and in Experiment 1A all nine soils were studied. Therefore, since more information is available about the effect of soil type on the soil and plant responses to the various treatments applied for soils 2 and 8, the following discussion will give slightly more weight to the responses of these two soils than to the responses of the other seven soils.

Soil pH

The pHs (measured in 0.01M $CaCl_2$) of the unameliorated soils ranged from 3.2 to 4.8 (median 3.7) (Table 3.2). The two highest pHs - 4.8 and 4.4 - were those of soils 3 and 9 respectively, the former soil was known to have been improved and it was thought that lime had

probably been applied to the latter. However, in all experiments except in Experiment 1 at lime level 0, the soils were limed to common pHs and although significant differences were frequently recorded between the pHs of different treatments, at the same lime level, the actual sizes of the differences were small (except for the between species difference, see Chapter III, Section 2.2). Hence the effect of pH would not have been a major factor contributing to treatment differences *within* a lime level.

The soil pHs were measured in 0.01M $CaCl_2$ (after Schofield and Taylor, 1955) rather than in distilled water as the former method gives a pH closer to that of the soil solution than the latter method - because the $CaCl_2$ is at a similar concentration to the soil solution which avoids the problems of exchanging cations (see Russell (1973) for discussion of this point). However, although $CaCl_2$ was used it was still the pH of the bulk soil that was measured and not that of the rhizosphere, i.e. the pH of the solution around the root surface, and it is the rhizosphere pH which will have a key influence on plant nutrient uptake. Nye (1981) has stated that rhizosphere pH may differ from that of the bulk soil, just a few millimetres away by 1-2 pH units; whether the difference is positive or negative will depend on the plant species being grown, the N source and the buffering capacity of the soil.

The buffering capacity of a soil is dependent upon the amount of exchangeable cations present in the soil, particularly H^+ and Al^{3+} and also on the amount of OM present since this will contain many potential sites for H^+/OH^- adsorption and exchange. The greater the buffering capacity of a soil, i.e. the higher its CEC and OM content, the more lime will be required to effect a given rise of pH and overcome the "available acidity".

Of the nine soils studied in Experiment 1A, soils 6 and 8 - the peat and the peaty podzol - required the greatest lime additions to raise their pHs from natural to about 5.0 - 17.4 and 11.1 mg lime kg^{-1} oven-dry soil respectively (calculated from Tables 3.2 and A1.1). The large lime additions were not a reflection of the low natural pHs of the soils, which were 3.0 for soil 6 and 3.4 for soil 8 (Table A3.1), since soils 4 and 7 which had natural pHs of 3.3 and 3.5 required only about a quarter to a half as much lime - 4.8 and 5.6 mg lime kg^{-1} oven-dry soil respectively.

On page 76 it has been noted that the lime additions made in Experiment 1A did not always achieve the "desired" pHs. The 'over-liming' precludes any similar comparisons to those above being made for the second lime increment but this 'overliming' was not the cause of the greater initial lime requirements of soils 6 and 8 - the pHs of soils 6 and 8 rose by 1.8 pH units whilst those of soils 4 and 7 increased by 1.5 units. Soils 6 and 8 must therefore have had greater buffering capacities than the other soils; this is what was expected as they had the highest OM contents of the nine soils studied - 83% and 41% loss-on-ignition respectively, and soil 8 also had the highest CEC - 171 meq 100 g^{-1} oven-dry soil (Table 3.2).

The form of OM and of clay present will affect the buffering capacity of a soil, as will the amount of Al^{3+}. This is because, particularly in the pH range 4.0 - 5.0, hydrolysis of Al^{3+} and polymerization of hydroxy-Al products may account for quite a large proportion of a soil's buffering capacity depending on how high its OM content is. Soils 1 and 5 both had very high extractable Al concentrations - 1050 and 1100 mg kg^{-1} oven-dry soil respectively; but since, excepting soils 3 and 9, soils 1 and 5 had the highest natural pHs of 3.9 and

thus needed relatively low lime additions, it was difficult to demonstrate from the information collected in Experiment 1 that they were well buffered.

Dry matter production

The discussion of the effect that soil type has on herbage DM production for PRG will be based on the results of Experiment 1A only (Figure 3.9) as the relative production from soils 2 and 8 in the other experiments is discussed in Section 6 of this chapter (IV). The results of the first harvest for the soils limed to level 4 will not be considered as all the soils were overlimed to some degree and consequently herbage DM production was reduced; only in soil 8 - the soil which was most seriously overlimed - was herbage production also reduced at the second and third harvests. Thus the initial setback in DM production was probably due more to slow establishment of the PRG plants in the alkaline soils because of delayed seed germination due to salt effects (high conductivity of the soil solution) and damage to the seedling roots: than to overliming *per se* that is induced minor and major nutrient deficiencies, Mn, Fe, Zn, B, Mg, P and K.

The relative DM production from the nine soils varied both with lime level and with harvest (Table A3.9). Of the unlimed soils, soil 7 produced the greatest amount of herbage at the first harvest and the second greatest at the second harvest, although it had a pH of only 3.5. This soil also produced the greatest amounts of DM at lime level 2 and at lime level 4, therefore under the conditions of Experiment 1A the combination of characteristics present in soil 7 gave the best DM production. The particular factor or factors present in soil 7 leading to this good production cannot be easily identified as although compared

to the other eight soils studied it had naturally high total N and extractable PO_4^{3-} contents (Table 3.2) fertilizer N, P and K additions were made to all soils in the experiment. However, it did have a relatively good structure compared to some of the soils studied and this could be a contributing factor.

The least production from an unlimed soil at all three harvests was obtained from soil 2 and, as for soil 7, the exact combination of factors present leading to the very low production cannot be distinguished. It is, however, very probably that the poor production was due to Al toxicity as the extractable soil Al content was 900 mg kg^{-1} oven-dry soil, and once the soil was limed and the lime had had time to act, DM production was considerably improved. At the final harvest at both lime levels 2 and 4, soil 2 produced the third most herbage.

Al toxicity could also have been limiting production from unlimed soil 1 and soil 5 (extractable Al content 1050 and 1100 mg kg^{-1} oven-dry soil respectively). Both these soils showed marked improvements in production with lime addition, and as for soil 2 the increased production was greater at the second and third harvests than the first - once the lime had had time to act. Thus soils 1, 2 and 5 together with soil 4 were generally the soils that exhibited the greatest DM response to liming.

The herbage production from soil 4 was in the middle of the range of DM productions achieved when the soils were limed, but was towards the bottom of the range of unlimed soils. Therefore, as for soil 2, there must have been a factor or factors present that limited production and was/were overcome with lime addition. It was not Al as the extractable soil Al content of soil 4 was only 350 mg Al kg^{-1} oven-dry soil, and although the soil had the lowest extractable K and PO_4^{3-}-P

concentrations of the soils studied, production was unlikely to have been limited as N, P, K fertilizer had been added. The factors responsible have not been identified, but it is probable that the improvements of soil structure brought about by liming was responsible for the increased production since the unameliorated soil 4 had a poor physical structure containing a lot of silt.

Although soil 8 was the soil worst affected by overliming being the soil that was most overlimed, it had the potential when limed to moderate pHs, as at lime level 2 (and lime level 3 in Experiment 1B) to produce reasonable quantities of herbage. Soil 8 was among the top three herbage producers at lime level 2 for the first two harvests.

The DM production from soils 3, 6 and 9 lay in the middle of the range of DM productions achieved and since the measured characteristics of the soils also lay in the middle of the ranges found, their relative DM productions are not discussed further.

Copper

The one treatment applied in Experiment 1A - lime addition - had little effect on the EDTA-extractable soil Cu content of soils 1 to 9 (Figure 3.3). Neither did P (Experiment 4) nor N addition (Experiment 5) affect the extractable Cu contents of soils 2 and 8, but the application of Cu (obviously) did (Experiment 5), as did the imposition of different soil water regimes in Experiment 6 [see Chapter III, Section 2].

The ranking of the nine soils with regard to EDTA-extractable soil Cu in the unameliorated soils (Table 3.2), and also in Experiment 1A (Table A3.3) was:

$$6 > 2 > 4 > 1 > 5 > 7 > 9 > 3 > 8$$

a range of 0.6 to 43.0 mg Cu kg^{-1} oven-dry soil with a mean content of 1.59 mg kg^{-1} (omitting soil 6). Soil 8, a peaty podzol with 41% LOI had the lowest extractable Cu content; the strong association between OM and soil Cu was reviewed in Chapter I, Section 3.1a. Surprisingly, soil 6, the peat with 83% LOI, contained the highest concentration of extractable soil Cu but since this soil was considerably less dense and more organic than the other soils studied the results of soil analyses are not strictly comparable - see Pimplaskar *et al.* (1982). The soil with the second lowest extractable Cu content was, however, that with the lowest LOI % - soil 3 with 10% LOI - presumably in this soil there was more Cu specifically adsorbed on soil clays and oxides than on OM. This apparent anomaly highlights the problems of trying to elucidate how soil Cu was behaving when only one form of Cu was assessed - Cu extractable by EDTA. If the time had been available, it would have been useful to both fractionate the soil Cu and to measure its total amount in order to assess better the soil physico-chemical relationships of Cu.

Recent evidence suggests that there are two types of sites involved in the soil adsorption of Cu - one set with high energy and another set with low energy which probably correspond to the sites of 'specific' and 'non-specific' adsorption respectively; and are to be found both in clays (Padmanabham, 1983) and OM (Lehmann and Harter, 1984). The proportion of Cu adsorbed on the high energy sites gradually increases with time, possibly because of alteration of the energy status of sites from low to intermediate and high (Abd-Elfattah and Wada, 1981; Padmanabham, 1983; Lehmann and Harter, 1984). Therefore, one would expect most of the small amount of Cu present naturally in soils to be held on at high-energy sites, i.e. to be specifically adsorbed

and not readily desorbable for plant uptake. McLaren *et al.* (1983b) states "it is clear that desorption or lack of desorption is very important in affecting plant availability of indigenous or added soil copper".

The concentrations of Cu found in PRG grown in the nine soils of Experiment 1A were not correlated to the EDTA-extractable soil Cu concentrations and, unlike extractable soil Cu contents, were affected by liming (Figure 3.11). The order of herbage Cu concentrations for the unlimed soils was basically

$$1 > 2 > 6 > 3 > 5 > 4 > 9 > 7 > 8$$

although the relative position of some of the soils, particularly soil 6, altered at each harvest. There was even more variation in the relative positions of the soils at lime level 4, possibly related to the initial very poor DM production, but soil 2 always produced the herbage containing the most Cu and soil 8 that with the least (Table A3.11) (soil 2 $\gg$ soil 8 was repeated in all the experiments). The order for the herbage grown in the soils was (meaned over the three harvests):

$$2 > 1 > 5 > 9 > 3 > 6 > 4 > 7 > 8$$

In 1982 a SAC/SARI study group compiled a provisional table for Scottish soils assessing the probable influence of soil association and series on Cu concentrations in summer herbage (see also Reaves and Berrow, 1984). The soils are grouped according to the total Cu contents of their B horizons (subsoils) into low, moderate and high risk soil series with regard to their ability to provide sufficient Cu to sustain normal growth and yield of cereal crops; hence the classes also assess the likelihood of a given soil series producing herbage with a low Cu concentration - which could affect the susceptibility of grazing animals to Cu deficiency. On this basis soils 1 and 4 are classified as low risk, soils 2, 7 and 8 as moderate risk and soils 3, 5, 6 and 9

as high risk soil series. It is therefore possible to produce a ranking of the soils with regard to the probability of them producing summer herbage with a low Cu content:

3, 5, 6, 9 > 2, 7, 8 > 1, 4

highest risk ⟶ lowest risk

This order is not very similar to the order of herbage Cu concentrations observed; for example, 2 and 8 instead of being at opposite ends of the list as was observed in Experiment 1A, are together in the middle, emphasizing that the SAC/SARI classification is provisional and has yet to be proven and finalized.

Although for Experiment 1A there was no correlation either between the initial extractable soil Cu concentrations and the herbage Cu concentrations or the soil and herbage Cu concentrations at the final harvest, there were correlations, mainly very highly significant ($p < 0.001$), between these measurements for the other experiments where fewer soils were studied (soil 2 and soil 8 had very different soil and herbage Cu concentrations). However, the situation in Experiment 1A is that more usually found, i.e. no correlation between extractable soil Cu contents and herbage Cu concentrations (e.g. Jarvis, 1981b). This lack of correlation arises for two basic (related) reasons: the first to do with plant nutrient uptake and the second to do with the method of measurement of soil Cu.

Plants obtain nutrients from the soil via their roots and thus only from that area of the soil which is directly in contact with their roots - the *rhizosphere*. Conditions in the rhizosphere are different to those in the bulk soil since roots exert an influence over their environment in many ways - for example, alteration of nutrient and

water gradients, modification of O_2 : CO_2 ratios via respiration, production of root exudates, stimulation of microbial activity and modification of pH (see Wilkinson, 1972; and Russell, 1977, for further discussions). Therefore it would be expected that the concentrations of nutrients in the rhizosphere would be different to those in the bulk soil, and Linehan has recorded this for Cu. Linehan (1984) demonstrated that the 'leakage'/exudation of low molecular weight organic molecules from root cells can lead to the mobilization of adsorbed Cu(II); and Linehan *et al.* (1985) observed higher soil solution Cu concentrations in the rhizosphere than in the bulk soil. The former (1984) observation could totally explain the latter (1985) but probably rhizosphere pH and soil differences will also have an effect (Smiley, 1974), as will microbial activity.

The method used for assessing extractable soil Cu status in this thesis was extraction by 0.05M EDTA (ammonium salt) at pH 7.0; as this is the method that is most commonly used in the U.K., e.g. by both ADAS/MAFF and the Scottish Colleges, which has been used for a considerable time (e.g. Mitchell *et al.*, 1957b; Blevins and Massey, 1959). The use of a different method would have, therefore, meant the results obtained here could not be meaningfully compared with published values.

EDTA is a chelating agent which extracts the complexed and chelated Cu^{2+} from the soil, however it is comparatively strong and was rejected by Lehmann and Harter (1984) for use in their desorption studies because it extracted "too much of the sorbed Cu"; and is stated by Williams and McLaren (1982) and McLaren *et al.* (1983a) to "overestimate soil Cu availability". Reasons why EDTA (and other extracting agents) do not adequately assess plant available Cu (nutrients), are discussed by Cox and Kamprath (1972), Viets and Lindsay (1973) and Robson

and Reuter (1981). The reasons suggested include: the extractant assesses bulk soil status, not rhizosphere status; there is no guarantee that the Cu extracted could be utilized by plants (and different species will have different needs); nor a guarantee that the method will consistently remove Cu from the same pools, for example, because of interference of other ions or OM; also equal concentrations of extractable soil Cu will not necessarily mean equal availability of Cu to plants. In Experiment 1A soils 1 and 5 both contained very similar amounts of EDTA-extractable Cu, e.g. 1.6 mg Cu kg^{-1} air-dry soil at the start of the experiment in the soil limed to *c.* pH 5.0 (Table A3.3), but herbage grown in soil 1 had a Cu concentration of 9.9 mg Cu kg^{-1} DM at the first harvest and that grown in soil 5 a concentration of 7.1 (Table A3.11).

Ideally a soil extractant should remove a nutrient from the same pools as a plant does, reflecting both the present availability of the nutrient in the soil and the ability of the soil to replenish the nutrient after plant uptake (Viets and Lindsay, 1973). Increasingly, weaker extractants are being recommended, for example DTPA, is the chelating agent frequently used in the USA, rather than EDTA (Baker and Amacher, 1982); and 0.1M $CaCl_2$ is being considered (McGrath *et al.*, 1985) for use, although it is not as successful for Cu as it is for Zn and Ni.

Molybdenum

The extractable soil Mo concentrations of the soils studied and the herbage Mo concentrations for plants grown in these soils were affected, to some degree, by all the soil treatments applied in the six experiments. The magnitude of the effect that a treatment had was

severely modified by the soil under consideration, for example soil 2 always produced a quite substantial 'Mo response', but soil 8 seldom produced a 'Mo response' of any size (e.g. Figures 3.22 and 3.50). The ranking of the soils with regard to their natural extractable Mo concentrations (Table 3.2) was:

$$6 > 8 > 2 > 7 = 9 > 3 > 4 > 5 > 1$$

with a range of 0.023 to 0.150 mg Mo kg^{-1} oven-dry soil and a mean of 0.0358 mg kg^{-1} (omitting soil 6). After the application of lime in Experiment 1A to raise the soil pH to 7 - 7.5 (i.e. lime level 4), the order was:

$2 > 6 > 5 > 8 > 7 > 9 > 4 > 1 = 3$ *start of experiment*

$2 > 6 > 5 > 8 > 7 > 9 > 3 > 1 > 4$ *conclusion of experiment*

For soil 5 in particular there was a considerable alteration in relative position with the addition of lime.

In 1982 a SAC/SARI study group compiled a table for Scottish soils assessing the probable influence of soil association and series on the Mo concentrations in herbage. The soils were grouped according to the total Mo content of their subsoils (B horizon) and their drainage status into low, moderate, high and very high risk series with regard to the probability of herbage growing in the soil accumulating "sufficient Mo to increase the risk of Cu deficiency in livestock". On this basis soils 2, 3, 5, 6, 7 and 9 are classified as low risk, soil 8 as moderate risk, soil 4 as high risk and soil 1 as very high risk series.

It would be therefore expected that the order of the soils with regard to herbage Mo concentrations would be:

$$1 > 4 > 8 > 2, 3, 5, 6, 7, 9$$

However, the rankings of the soils with regard to herbage Mo concentrations in Experiment 1A (meaned over the three harvests) were:

$$3 > 9 > 7 > 1 > 2 > 4 > 8 > 6 > 5 \quad \textit{unlimed}$$

$$2 > 3 > 5 > 1 = 9 > 4 = 6 > 8 > 7 \quad \textit{lime level 4}$$

and the order of 'responsiveness' (size of increase lime level 0 to 4) was:

$$5 > 2 > 6 > 1 > 9 > 7 > 4 > 3 > 8$$

None of these rankings bears much relationship to the order expected from the SAC/SARI assessment. For example, the herbage grown on the very high risk soil-number 1 was not greatly different in Mo concenration or responsiveness to many of the other soils, i.e. it lay in the middle of the concentration and response ranges. The two soils which generally supported the herbage with the largest Mo response to liming, and were among those giving the highest herbage Mo concentrations were soils 2 and 5 - low risk series - yet soil 7 also a low risk series generally produced herbage containing among the lowest Mo concentrations, that were little altered by lime application. This serves to emphasize that the "classification must be regarded as provisional and subject to future revision as additional data on herbage Mo and its relationship to the incidence of Cu deficiency in livestock become available" (SAC/SARI, 1982). It must not, however, be forgotten that it is not only the propensity of a soil to support herbage with high Mo concentrations, particularly after hill land improvement, that is used to assess the risk status of a soil series with regard to the ability to produce an induced Cu deficiency in grazing livestock, but also the relative balance of herbage Cu, Mo and S concentrations (see Section 7 of this chapter).

When correlation analyses were performed between the soil and herbage Mo concentrations the relationships were not found to be as significant as they had been for soil and herbage Cu for soils 2 and 8 (see page 192). The extractable soil Mo contents at the outset of an experiment and the herbage Mo concentrations were not significantly correlated for Experiment 1A (second and third harvests), Experiment 5 (both harvests) and Experiment 6, but were significantly correlated at $p < 0.05$ for the first harvest of Experiment 4 and at $p < 0.01$ for the first harvest of Experiment 1A, all three harvests of Experiment 1B and the second harvest of Experiment 4. When the extractable soil Mo and herbage Mo contents at the final harvests were related for these five experiments (1A, 1B, 4, 5 and 6), there was no correlation for Experiments 1A, 1B, 4 and 5 but a correlation significant at $p < 0.05$ for Experiment 6 which had only one harvest. However there was a very highly significant correlation ($p < 0.001$) between herbage and extractable soil Mo concentrations at the first harvest of Experiment 1B. It therefore appears that where fewer soils were present in the analysis a greater degree of correlation was found - soil 2 contained large amounts of extractable soil Mo and responded greatly to most treatments and produced herbage with high Mo concentrations, whereas soil 8 which also contained large amounts of extractable soil Mo was the opposite; and that correlation is better early in plant growth experiments rather than towards the end.

The points raised in the discussion on page 192 for Cu also apply to Mo, that is: differences between rhizosphere and bulk soil and the problems of correctly assessing how much nutrient is available for plant uptake. The latter is particularly difficult for Mo as soil Mo availability is generally considerably affected by pH (e.g. Figure

3.5); also there are only very small amounts present in the soil which until comparatively recently were more or less impossible to accurately assess, and even with more modern techniques it is still not easy to measure soil Mo (of any form).

The extraction method used for the soils was N NH_4OAC at pH 7.0 which will assess adsorbed Mo (from OM, clays and oxides), and water soluble molybdate from the soil solution. Not all of the adsorbed Mo will be extracted, the proportion extracted will depend upon how strongly and where the Mo is held, and also on other soil factors. Molybdenum can absorb onto both positively and negatively charged surfaces in the soil as it is present in both anions and cations (see pages 14 and 19), so there is a surprisingly close relationship between the amounts of OM and molybdenum in soils.

The extractable Mo concentrations of soils 2 and 5, the two most Mo responsive soils to lime, were before the application of any treatments 0.028 and 0.042 mg Mo kg^{-1} oven-dry soil and the concentrations of soils 7 and 8, two soils of low response, were 0.038 and 0.049 mg Mo kg^{-1} oven-dry soil respectively. Therefore, the degree of Mo-response of the soil studied appears unrelated to their initial extractable Mo contents. If Table 3.2 is studied it is seen that there is not really a common difference between soils 2 and 5, and 7 and 8, the only real differences are that the former have higher extractable Al concentrations and the latter higher extractable Mg concentrations. But since both Al and Mg are present as cations in the soil and Mo is present predominantly as an anion this is unlikely to be the cause of the different behaviour of the soils, rather it is probably related to a factor or a combination of factors that was not measured. It is possible, however, that the greater Al concentration reflects the presence of more Al oxides which are known to absorb Mo (Parfitt, 1978).

The increase in soil Mo availability following liming is attributed to anion exchange between OH^- and MoO_4^{2-} (Fleming, 1980), so it is possible that there are more adsorptive surfaces present in the more responsive soils - particularly Mn, Al and Fe oxides. If this is so the combination of less adsorptive surfaces together with more OM for soil 8 (but not soil 7), where Mo can be held, will mean less soil 'Mo response', since Mo in combination with OM is less affected by pH than 'inorganically' adsorbed Mo (Bloomfield and Kelso, 1973). In order to ascertain whether there are more potential sites for Mo adsorption and anion exchange present in the more Mo-responsive soils, it would be necessary to establish the types and proportions of the different soil clays and oxides present and to assess their relative adsorptive capacities.

There is evidence (e.g. Mitchell, 1964; Fleming, 1980) that micro-organisms may also play a greater role in soil Mo availability than was previously thought, both by encouraging Mo uptake and by increasing Mo availability by fixation of Mo on microbial organic complexes rather than on other soil components. It is possible that there are different types of micro-organisms present in the two classes of Mo-responsive soils.

Sulphur

The extractable SO_4^{2-}-S concentrations in the soils studied and the herbage S concentrations of plants grown in these soils were modified by the treatments applied in the six experiments. Although both S and Mo are present in the soil predominantly as anions (Mo can also exist as a cation) and undergo similar reactions, the magnitude of the responses to the applied treatments was less for S than it was for Mo

both within soils and between soils (i.e. relative and absolute). For example, whereas soils 2 and 5 when limed produced the greatest increases in herbage Mo concentration, they produced the smallest increases in herbage S concentrations.

The order of ranking of the extractable soil SO_4^{2-}-S contents of the unameliorated soils was:

$$6 > 5 > 1 > 2 > 8 = 7 > 9 > 4 > 3$$

with a range of 9 to 66 mg SO_4^{2-}-S kg^{-1} oven-dry soil and a mean of 29.3 mg kg^{-1}. This order was basically unaltered in the unlimed soils of Experiment 1A where only basal NPK had been added, but was changed when lime was also applied (Table A3.7). Soil 8, in particular, showed a large change in its relative position after liming because of a large increase in extractable soil SO_4^{2-}-S contents at the end of the experiment; but if the results of Experiment 1B (Figure 3.8 and Table A3.8) are studied, it is seen that nearly all this increase occurred between levels 3 and 4, i.e. pH 6 and 7.5 - the heavily overlimed treatment. If the results of the other experiments are considered it is seen that at pHs $\leq$6.5, the extractable SO_4^{2-}-S content of soil 8 was generally less affected by the imposed treatments than that of soil 2 (e.g. Figures 3.35 and 3.51).

It was difficult to discern which of the soils exhibited the greatest increase in extractable soil SO_4^{2-}-S in Experiment 1A (Figure 3.7) as the responses produced varied with the amount of lime applied and with the time of sampling, but soils 3 and 7 consistently gave the lowest increases. As for the other elements studied (Cu and Mo) it is difficult to propose a reasonable hypothesis for the differing responses of the soils when only one form of analysis was used for the element in question;

and the proportions and types of different adsorbing surfaces present in the soil (oxides, clays, OM) are not known. Soil 3 did naturally contain the least extractable SO_4^{2-}-S but soil 7 was in the middle of the range of contents at 18 mg SO_4^{2-} kg^{-1} oven-dry soil, which was a similar content to soil 4 (16 mg SO_4^{2-} kg^{-1} oven-dry soil), yet soil 4 exhibited one of the biggest responses to liming.

Where correlation analyses were carried out between soil and herbage S contents at the same harvest, they were always found to be significant: $p < 0.01$ for Experiment 1B first harvest and Experiment 4 final harvest, and $p < 0.001$ for the second harvest of Experiment 1B and the final harvests of Experiments 1A, 1B, 5 and 6B. Correlation analyses were also carried out between the initial extractable soil SO_4^{2-}-S contents and herbage S concentrations and were found to be: non-significant at the first harvest for Experiment 1B and both harvests of Experiment 5; $p < 0.05$ at the first harvest of Experiment 1A, the second harvest of Experiment 1B, both harvests of Experiment 4 and the one harvest of Experiment 6B; and $p < 0.001$ at the second and third harvests of Experiment 1A and the third harvest of Experiment 1B. For the experiments in which two species were grown it was observed that PRG herbage S concentrations were better correlated with extractable soil SO_4^{2-}-S contents than were WC herbage S contents. The extractant used for the SO_4^{2-}-S was KH_2PO_4 which extracts the readily soluble sulphate plus some adsorbed SO_4^{2-} as well as a little organically adsorbed SO_4^{2-} - PO_4^{3-} extractants are considered to give good correlations between soil S extracted and plant S uptake (Tsuji and Goh, 1979). But the considerations of the differences between rhizosphere and bulk soil and the limitations of any soil extractant, as discussed on page 192, apply to SO_4^{2-} as well as for Cu and Mo.

The ranking of the soils with regard to the S concentration of plants grown in them altered as the amount of lime applied increased and also with the harvest under consideration (Table A3.15). The order of ranking of the soils with regard to the relative size of the response in plant S concentrations (unlimed soil compared to soil at lime level 4) was, however, more or less the same at each harvest and was bascially:

$$8 > 9 > 7 > 6 > 3 > 4 > 1 > 2 > 5$$

although the relative position of soils 6, 7, 8 and 9 did alter at each harvest.

The three soils that produced the plants showing the least response in plant S concentrations after liming were those with the highest extractable Al contents when unameliorated (Table 3.2) and consequently the soils which produced the greatest increase in DM production after liming (Table A3.9). It is therefore possible that the lack of response was a 'dilution effect', i.e. although the amount of S taken up was enhanced by liming, so was DM production, and consequently there was little effect of liming on herbage S concentrations.

The mean amounts of S in the herbage grown in each soil at the different lime levels for the three harvests of Experiment 1A are given in Table 4.1 and it can be calculated that the order of 'responsiveness' was:

$$2 > 4 > 5 > 9 > 6 > 7 > 1 > 3 > 8$$

That is, when amounts of herbage S were considered soils 2 and 5 did show relatively large increases following liming, although soil 1 did not.

Table 4.1: Amount of S present in PRG herbage grown in soils 1 to 9 at 3 lime levels - mean amount for three harvests.

Soil	*Amount S taken up by PRG herbage (mg pot^{-1}) (mean of three harvests)*		
	Unlimed	Lime level 2	Lime level 4
1	2.26	3.19	4.34
2	0.49	3.50	2.83
3	1.15	1.19	2.07
4	1.31	3.31	4.05
5	1.36	3.73	3.61
6	1.47	2.02	3.00
7	1.89	3.43	3.83
8	1.37	2.55	0.29
9	1.85	2.06	3.97

The behaviour of the amounts of S taken up by PRG grown in soil 8 was analagous to that in soils 2 and 5, i.e. it also moved from being at one end of the ranking to the other. However, it is difficult to interpret the results for soil 8 as lime level 4 was considerably over-limed with consequent release of SO_4^{2-}-S by anion exchange on hydrous oxides (Harward and Reisenauer, 1966) and mineralization (Williams, 1967b) which led to increased plant herbage S concentrations but greatly reduced DM production.

As already discussed, it is not easy to explain the relative level of response produced in and on any of the soils; and the remaining soils are more or less indistinguishable from each other in degree of response and amounts of S present (in both soil and herbage).

2. "DRAINAGE"

The effect that soil water content has on the Cu, Mo and S content of soils and of plants grown in the soils was investigated in Experiment 6 - soils 2 and 8 were incubated at different moisture contents for three months (Part A) and plants were then grown in the pre-treated soils (Part B). The results are given in Chapter III, Section 2.6.

Copper

(a) Soil incubation

The EDTA-extractable soil Cu concentrations of soils 2 and 8 were relatively unaltered by three months moist incubation with and without added lime (Figure 3.49). A decrease in EDTA-extractable Cu was noted by Williams (1981) for ten Scottish soils following a ten-month moist incubation. However, since all ten soils studied were from

lowland sites their properties were very different from hill soils 2 and 8 used in this work, e.g. higher natural pHs and EDTA-extractable Cu contents and lower OM contents.

Waterlogging of soil 8 did not alter the EDTA-extractable Cu content (Figure 3.49b) but for soil 2 produced a large initial decline followed by a small recovery (Figure 3.49a). Ng and Bloomfield (1962) have also observed extractable soil Cu contents to decline with waterlogging. Although Cu does not itself undergo any chemical changes when reducing conditions develop in a soil following waterlogging, its availability will change because of: microbial immobilization, increased Cu-OM complexation as soil OM is solubilized and broken down, and increased adsorption on newly exposed oxide surfaces (particularly Mn) (e.g. Basak *et al.*, 1982; Haldar and Mandal, 1979).

Liming the soil prior to waterlogging did not modify the effect of waterlogging on extractable Cu concentrations for either soil, although it might be expected to intensify the reducing conditions and bring about any changes more rapidly (e.g. Ng and Bloomfield, 1962; Iu, 1981). (The decline in extractable Cu in soil 2 with waterlogging did proceed marginally more quickly when lime was present.) Reducing conditions are also believed to be intensified by high OM contents but the extractable Cu content of the more organic soil, number 8, was not affected by waterlogging in contrast to the results of Haldar and Mandal (1979) - although they added OM to a rice soil (high pH) rather than using a soil with a naturally high OM content and low pH.

Iu (1981) examined the behaviour of the different soil Cu fractions to waterlogging and observed that the soluble, exchangeable and organically bound Cu decreased whereas the specifically adsorbed and oxide adsorbed Cu increased. Hence the relative balance of these fractions

in a soil will determine how its Cu content is affected by waterlogging. Since fractionation of the soil Cu was not possible in the work reported in this thesis, a full explanation for the difference in behaviour of the extractable Cu contents of soils 2 and 8 cannot be given.

(b) Plant growth

The EDTA-extractable soil Cu content of both soil 2 and soil 8 was unaffected by the two watering regimes (60% and 100% FC) (Table 3.14) as was the herbage Cu concentration of the PRG grown in soil 8 (Figure 3.54b). The herbage Cu content of PRG grown in soil 2 was, however, less when PRG was grown under the wetter conditions (Figure 3.54a). There was greater herbage DM production (Table 3.17) in the wetter treatment for both soils but the relative increase was greater for soil 2 so the difference noted above could be a dilution effect (see page 30).

For both soils the waterlogging pre-treatment resulted in higher extractable soil Cu and plant Cu contents than the dry and moist pre-treatments, despite only soil 2 exhibiting an effect on extractable Cu contents (a decrease) with waterlogging during the soil incubation (see above). However, the waterlogged soils from the incubation had been dried out slightly between the two parts of Experiment 6 (in order for plant growth to be possible) and during this time reoxidation of the soils had probably occurred with associated breakdown of soil oxides and release of adsorbed Cu. The extractable Cu content of soil 8 approximately doubled between Part A and Part B of the experiment and that of soil 2 increased five-fold. An increase in plant Cu content following soil waterlogging has also been recorded by Iu *et al.* (1982) for French bean (but not maize) and Berrow *et al.* (1983) for pastures.

Molybdenum

(a) Soil incubation

Neither waterlogging nor lime addition had an effect on the extractable Mo content of soil 8 (Figure 3.50b), but both treatments increased the amount of Mo extractable from soil 2 and their effect was additive (Figure 3.50a). Although it is widely accepted that the extractable Mo content of soils is increased by poor drainage, there is little published evidence but two examples are Ng and Bloomfield (1962) and Mitchell (1964). The results from Experiment 6A suggest that soil type has a strong influence on the effect of waterlogging/poor drainage on extractable soil Mo concentrations.

The increased extractable Mo content of soil 2 is attributable to the release of molybdate from soil oxides and OM, which broke down when reducing conditions developed. The increase of soil pH in the waterlogged soils (due to lime addition and/or the effects of waterlogging - Figure 3.48a) will have encouraged the adsorption of PO_4^{3-} rather than MoO_4^{2-} and hence have contributed to the increase in extractable soil Mo (see page 215).

(b) Plant growth

The moisture regime at which the soils were maintained during plant growth did not affect the extractable soil Mo concentration of either soil (Table 3.15) or the Mo content of plants grown in soil 2 (Figure 3.55a); but for soil 8 the wetter regime resulted in lower plant Mo contents (Figure 3.55b). In contrast to the plant Cu concentrations there was therefore no evidence of a dilution effect in soil 2 but there was evidence in soil 8; differences in the plant uptake mechanisms for Mo (an anion) and Cu (a cation) could explain this anomaly.

Soil 2 which had been waterlogged as a pre-treatment contained more extractable Mo than the moist or dry pre-treated soil, although there was two-thirds less Mo extractable at the start of Part B than at the end of Part A, i.e. reoxidation of the soil as it dried, led to readsorption of some of the Mo released by waterlogging. However, there was also a decrease in the Mo content of the moist incubated soil between the two phases of the experiment although neither soil pH nor amount of water present altered.

The extractable Mo content of soil 8 had not been affected by waterlogging during the incubation and likewise when used for Part B the soil contained similar amounts of Mo to that which had been incubated moist. The Mo content of the incubated soils (moist and waterlogged) decreased between the two phases of the experiment as had also occurred for soil 2, but to a lesser extent, possibly because of the more organic nature of the soil. Ng and Bloomfield (1962) suggest that the presence of OM inhibits coprecipitation of Mo with ferric oxides formed on re-aeration of waterlogged soils. It had been expected that the soil which had been stored dry might contain less extractable Mo at the start of Part B than either of the incubated soils because the Mo would still be adsorbed; but it contained more. This was probably because water and lime had only recently been applied and thus microbial activity and nutrient release had been encouraged (the 'Birch' effect: see page 21).

The waterlogged pre-treated soil 2 contained more extractable soil Mo than the other two pre-treatments but PRG grown in this soil contained less Mo. This is consistent with the observations of Davey (1957) but is the reverse of the general accepted relationship (e.g. Kubota *et al.*, 1963). For soil 8 the pre-treatment which contained the

most extractable Mo supported the plants containing the most Mo, i.e. the dry pre-treatment. The dry pre-treatment had also supported the plants with the greatest Mo concentrations for soil 2. It is possible that the moist/waterlogged incubation of the soils produced a compound, for example high concentrations of ethylene (Dowdell *et al.*, 1972), which both reduced plant production (Table 3.17) and interfered with plant Mo uptake.

Sulphur

(a) Soil incubation

Waterlogging of soil 2 and soil 8 produced a decrease in the concentration of extractable soil SO_4^{2-}-S which was more dramatic in soil 2 than soil 8, and occurred less rapidly where lime had been applied (Figure 3.51). The decrease in SO_4^{2-}-S is a consequence of microbial activity; the obligate anaerobes *Desulfovibrio* and *Desulfotomaculum* use SO_4^{2-} as an electron acceptor during respiration and reduce it to S^{2-}. The S^{2-} is generally given off from the soil as H_2S (this was smelt in Experiment 6A) or other volatile sulphides, e.g. dimethyl sulphide (Lovelock *et al.*, 1975) but may remain in the soil as HS^- or as metal sulphide precipitates, e.g. FeS (Ponnamperuma, 1972, 1984).

Ponnamperuma (1972, 1984) has described an initial rise in extractable soil SO_4^{2-}-S contents in acid soils prior to the decrease discussed above; the increase is due to desorption of SO_4^{2-}-S from soil clays and oxides (*cf.* Mo). There was some evidence for an increase in soil 2 when it was unlimed but not in the more organic soil 8.

(b) Plant growth

The soils which were maintained at 100% FC contained less extractable SO_4^{2-}-S than the soils maintained at 60% FC and correspondingly

plants grown in these soils had lower S concentrations (Table 3.16 and Figure 3.56). A dilution effect (the wetter soils produced more herbage) could have also contriubted to the lower plant S concentrations. Williams (1975) observed that herbage on poorly drained soils contained less S than herbage grown in well drained sites, although the situation was reversed with regard to extractable soil SO_4^{2-}-S contents.

For both soils the order of SO_4^{2-}-S content with regard to pre-treatment was moist > dry > waterlogged. The content of SO_4^{2-}-S was unaltered between Parts A and B of the experiment for the moist treatment but that of the waterlogged had increased, presumably because SO_4^{2-}-S was reformed as the soil was aerated and reoxidized. The moist pre-treated soils contained more SO_4^{2-}-S than the dry pre-treated soils, probably because the lime had been present for longer and this outweighed the initial flush of nutrient release when the dry soil was rewetted. There was no consistent pattern in the effect of pre-treatment on herbage S concentrations; those of PRG grown in soil 8 were the same as the order of extractable soil SO_4^{2-}-S but this was not so for soil 2 (Table A3.52). However, there was a strong correlation ($p < 0.001$) between the soil and herbage S contents at harvest (considering both soils together).

3. LIMING

The discussion of the effect that liming has on the soil-plant relationships of Cu, Mo and S will be restricted to the results of Experiments 1 and 2; the results of Experiment 4 - the lime x phosphorus trial - are discussed in Section 4.1 of this chapter.

Copper

EDTA-extractable soil Cu contents were relatively unaffected by small increases in soil pH but larger increases in pH, especially in the range pH 5-7, slightly increased soil Cu contents for most of the soils studied (Figure 3.3). No significant correlations were found between soil pH and soil Cu contents. An increase in soil solution Cu concentration as the pH of a soil was increased was observed by McLaren *et al.* (1981) and was accounted for by increased solubilization of OM as the pH increased, leading to increased Cu-OM complexation (Sanders and Bloomfield, 1980; McLaren *et al.*, 1981, 1983a). Sanders (1982) has observed that the amount of free Cu^{2+} ion in the soil solution decreases dramatically as soil pH is increased, which he attributed to increasing complexation. Liming stimulates the mineralization of OM (Ellett and Hill, 1929) and this may lead to the release of Cu. Decreases in soil Cu content as pH is increased have frequently been noted in work earlier than that of McLaren and Sanders cited above (e.g. McLaren and Crawford, 1973b), the decreases were accounted for by increased Cu sorption on soil oxides. Such work was, however, performed at unrealistically high solution Cu concentrations rather than at the low Cu concentrations found naturally in soils where only a small percentage of possible adsorption sites for Cu are occupied (Williams, 1981).

A decrease in extractable soil Cu content when the pH was increased was observed for soil 6 (Experiment 1A) and for soil 8 (Experiment 1B). Soil 8 was a peaty podzol (41% LOI) which contained the least soil extractable Cu of the nine soils studied and the decrease was small, but soil 6, the peat, (83% LOI) contained the most Cu and the decrease was quite large. It might be expected that in such organic soils the formation of soluble Cu-OM complexes at high pH would outweigh

the increased adsorption of Cu, as was the case in the other soils, but this did not happen. The results for soil 6 (a peat) must be treated with caution as they are not directly comparable to those for the other soils because of the very different weight to volume ratios (see Pimplaskar *et al.*, 1982). A possible explanation for the small decrease in extractable Cu content that occurred in soil 8 could be competition between Cu and Ca for complexation with the newly solubilized OM - soil 8 was the soil that received the largest lime additions (Table A1.1).

The pHs of the soils in this study ranged from 3.0 to 7.8 (with natural pHs of 3 to 5 - most <4), whereas most other studies have been carried out in the pH range 5 to 7, and on soils that have 'field' pHs in this range - lowland agricultural soils. McLaren *et al.* (1983a) observed a lack of response of EDTA-extractable soil Cu to pH between 4.5 and 6.5 but decreasing extractable soil Cu as pH increased from 3 to 4.5 and increasing extractable soil Cu as pH increased from 6.5 to 7.5. However, in the work presented here, this pattern of response was observed only in soil 2, which was a brown forest soil as was the soil studied by McLaren. None of the other 8 soils, including soil 7, a brown forest soil from the same series - Sourhope - as McLaren's soil, followed the pattern. The 'field' soil pHs of all the soils studied were also lower by 1 to 3 pH units than those of McLaren's soils. It is possible that the extractable Cu content of a soil does not vary greatly around the soil's natural pH as an equilibrium exists between Cu held on all the different soil components; thus as pH is altered adsorption or desorption of Cu readily occurs since so few sorption sites are occupied.

The application of lime did not have a consistent effect on plant Cu concentration; the effect varied with soil, amount of lime applied, plant species and harvest, i.e. the length of time since lime was applied to a soil. For the nine soils studied in Experiment 1A there was generally no effect of liming on Cu concentration of the plants after 35 days (although soil 5, a brown forest soil containing 1.6 mg Cu kg^{-1}, exhibited an increase). By 60 days for most soils herbage Cu content decreased as the amount of lime that had been applied to the soil increased, and by 90 days PRG grown in all soils exhibited decreased Cu content with liming; the decrease was greater than that at the second harvest (Figure 3.11).

A similar pattern of results was observed in the more detailed study of Experiment 1B. After 35 days liming of the soils had had no effect on plant Cu concentration and by 60 days there was slightly more evidence of an effect of liming, but this was only really exhibited as a decrease in the Cu content of PRG grown in soil 4. By 90 days plants grown in all three soils showed decreased Cu content with lime application and for soil 4 the magnitude of the decrease was greater than at 60 days (Figure 3.12). Correlation analyses between soil pH and soil Cu content at each harvest were non-significant at the first two harvests but highly significantly negatively correlated ($p < 0.001$) at the third harvest, reflecting the pattern of results described above.

There were only two harvests taken in Experiment 2, at 40 and 70 days; for PRG the results were the same as in Experiment 1B, i.e. no effect of liming on plants grown in any of the three soils at the first harvest, and a decrease for plants grown in soil 4, only, at the second harvest (Figure 3.20).

Although soil type definitely influenced the response of plant Cu concentration to liming, it did not consistently have the effects reported by Burridge *et al*. (1983), i.e. that on Cu deficient soils small decreases in herbage Cu occur with lime addition whilst on Cu adequate soils there is no effect, or a small increase. Soil 8 may be classed as Cu deficient according to SAC/SARI (1982) (the threshold for Cu deficient soils is set as 0.8 mg Cu kg^{-1} air-dry soil for a mineral soil of pH 6.0 - soil 8 is a peaty podzol with pH 3.5), but although it did give lowered herbage Cu concentration with lime application, larger decreases were found in soil 4 which contains 2.1 mg Cu kg^{-1}.

An indication of the modifying influence that soil has upon the effect of liming on plant Cu uptake can also be found from the correlation between plant and soil Cu contents in Experiment 1. In Part A there was no correlation between plant and soil content but in Part B correlations both between initial extractable soil Cu and herbage Cu at the harvests and soil and herbage Cu at each harvest, were highly significant ($p < 0.001$). The apparent difference between the two parts of the experiment was probably brought about because there were more soils in the correlation for Part A and each soil modified the plant response slightly differently, whereas in Part B the three soils, despite being very different (see Section 1, this chapter) all brought about similar patterns of decrease in plant Cu content.

The response of WC to liming in Experiment 2A was basically the same as for PRG but the response appeared to develop more rapidly, possibly because of the greater Cu content of WC (Section 5, this chapter). For all three soils there was no response in the Cu concentration of WC to liming on day 40, but by day 70 there were decreases for WC growing on the more highly limed treatments for soils 4 and 8 and a

slight increase in the Cu content on soil 2 (Figure 3.20). Clover was also observed to respond more than grass to liming by Mitchell *et al.* (1957b).

There was no difference observed in Experiment 2B in the response of the Cu content of PRG to liming whether the lime had been applied to all the soil in the plant pot or just to the top 25% (by volume) of the soil (Figure 3.21). It therefore seems improbable that the effect of liming on plant Cu contents was mediated via reduced H^+ ion concentrations *per se* [reducing acidity can be associated with decreased competition for uptake and reduced root damage (Graham, 1981)]; but rather via increasing Cu-OM complexation in the soil reducing plant Cu availability.

Molybdenum

It is widely accepted that liming an acid soil will increase Mo availability due to Mo release both from soil oxides, as OH^- and $H_2PO_4^+$ are preferentially adsorbed, and from mineralized OM; and that the magnitude of this effect will be modified by soil (e.g. Fleming, 1980). The results obtained in Experiment 1 confirm this: the amount of extractable Mo in all soils, except number 4, increased with lime addition and the effect was particularly large for soil 2 (Figure 3.6), where a 5-fold increase occurred between pH 3.5 and 7.0. The degree to which extractable soil Mo levels increase with the application of lime will vary depending on the amount of soil oxides, HCO_3^--ions, OH^--ions and OM in the soil. When soil pH at harvest was correlated with soil extractable Mo content, for Experiment 1B the degree of correlation decreased as the experiment progressed from being very highly significant ($p < 0.001$) at the first harvest to significant ($p < 0.005$) at the second and non-significant at the third.

Generally, plant Mo concentrations increased as pH increased, as also observed by Mitchell *et al*. (1957b); but as the period of time for which the lime had been applied lengthened so the size of response decreased (Figure 3.13), particularly at higher pHs, although soil extractable Mo contents remained high. The magnitude of the effect of liming on herbage Mo content varied with the soil but was greatest in those in which extractable Mo levels had been the most affected, notably soil 2, and hence in which more molybdate was available for plant uptake.

This was well illustrated in Experiment 1B (Figure 3.14): whereas the Mo content of PRG growing in soil 2 was greatly affected by liming at all three harvests, the effect was only present at the first harvest for soil 4 (the extractable Mo content of soil 4 was unaffected by liming) and at the first two for soil 8. The same pattern was also observed in Experiment 2A (Figure 3.22). Despite the difference in response of the soils at each harvest there was a significant correlation ($p < 0.05$) between soil pH and herbage Mo content at each harvest.

Correlation analyses were carried out between initial extractable soil Mo content and herbage Mo content at each harvest. Whereas the correlation was highly significant ($p < 0.01$) for all harvests in Experiment 1B, in Experiment 1A where more soils were studied only the correlation for the first harvest was significant. Correlation analysis was also performed to see if there was any relationship between herbage Mo content and the soil Mo content at the harvests in Experiment 1B. There was a very highly significant correlation ($p < 0.001$) on day 35 but no correlation on days 60 or 90.

In Experiment 2A both WC and PRG were grown and although both species contained similar mean amounts of Mo after 40 days, WC,

unlike PRG, responded to lime application at the top level in soils 4 and 8, in addition to the response in soil 2 (Figure 3.22a). The latter response was much greater than that for PRG. By day 70 both species reacted similarly to liming, a response only occurring in soil 2 (Figure 3.22b). Mitchell *et al.* (1957b) also observed a greater response of plant Mo content to liming in clover than grass. The greater response of the Mo content of WC to liming than that of PRG was probably partly due to increased nitrogen fixation stimulated by the elevated levels of soil Mo but could also have been due to the different cation/anion balance of the two species.

Although the method of lime application had no effect on the Mo concentration of PRG at the first harvest in Experiment 2B (Figure 3.23a) it did at the second (Figure 3.23b). For soils 4 and 8 the plants grown in pots with surface applied lime contained less Mo than the plants in pots with lime applied throughout the soil. The pH of the surface soil (where there would have been many plant roots) would have been greater in the former pots, and Stout *et al.* (1951) have observed a decreased ability of plants to absorb MoO_4^{2-} as pH increases. Since by analogy with Experiment 1 it is believed that the extractable soil Mo contents of these soils (4 and 8) did not alter with liming, plant Mo content consequently decreased. For soil 2 at the lower pHs, i.e. at lime levels 1 and 2, PRG grown in pots with surface applied lime contained more Mo than PRG in pots with lime applied throughout the soil. This observation is consistent with the response of extractable soil Mo shown by this soil to liming; but at lime level 3 the situation was as for soils 4 and 8, with plants grown in pots with surface applied lime containing less Mo than plants grown in pots to which lime had been applied throughout the soil. This result could have been related

to the poorer growth in the former pots together with the decreased absorptive capacity of the plants for Mo as pH increased.

Sulphur

The rise in soil pH from *c.* 5.0 to 7.5 greatly increased the amount of extractable soil sulphate in all soils, except soil 3 (which had already been limed) and soil 7. The magnitude of the effect was greater at about 100 days after liming than about 10 days after (Figure 3.7d,a). A rise in pH from *c.* 3.5 to 5.0 did not affect the extractable soil SO_4^{2-}-S content. The more detailed study in Experiment 1B (Chapter II, Section 2.1) of soils, 2, 4 and 8 confirmed that soil SO_4^{2-}-S content increased with liming, that the increase was greater nearer pH 7.0, and that the magnitude of the increase was greater the longer a soil had been limed. There were significant correlations between soil SO_4^{2-}-S contents and soil pH at the time of all three plant harvests in Experiment 1B, $p < 0.001$ for the first and third harvests, and $p < 0.01$ for the second.

Increases in the extractable soil SO_4^{2-}-S contents of soils after lime application are normally observed (e.g. Kamprath *et al.*, 1956) and arise for several reasons: raised pH encourages competition for sites of adsorption on Fe and Al oxide between OH^- and SO_4^{2-} (Hingston *et al.*, 1972), increases competition for adsorption between SO_4^{2-} and MoO_4^{2-} and $H_2PO_4^{2-}$ (whose solubilities are increased as pH rises) (Metson and Blakemore, 1978), increases mineralization rates (Williams, 1967b) and increases the solubility of sparingly soluble hydroxysulphates (Wolt, 1981).

Liming generally increased the S concentration of plants growing in the soil (see pages 101 and 120). There was no marked difference

in response whether WC or PRG were being considered (Figure 3.24). Liming a soil is normally observed to increase plant S content (e.g. Elkins and Ensminger, 1971) as extractable soil SO_4^{2-}-S concentrations and hence SO_4^{2-}-S availability are increased for the reasons discussed above.

The magnitude of the effect that liming had on plant S content decreased during the course of both Experiments 1 and 2. Yet, whereas soil pH and herbage S for Experiment 1B were not correlated at either the first or second harvests, there was a highly significant correlation at the third ($p < 0.01$). Hue *et al.* (1984) studied a S-deficient Ultisol which had been limed in a glasshouse experiment to increase SO_4^{2-} availability, and observed that the initial increase in soil S availability brought about by liming (which was reflected in increased yields of wheat growing in the soil), did not continue beyond about 35 days. This effect was attributed to the large initial plant S uptake, leading to depleted soil available S levels. Although this is also a possible explanation for the observations in Experiments 1 and 2, none of the soils studied were S deficient and in Experiment 1 it was noted that soil SO_4^{2-}-S levels continued to be higher at high pHs than at low pHs, throughout the experiment and that the magnitude of this effect increased with time.

In Experiment 1A herbage S% and soil SO_4^{2-}-S content at the third harvest were found to be very highly significantly correlated ($p < 0.001$), as was the case at the second and third harvests in Experiment 1B, but there was no significant correlation for the first harvest in Experiment 1B. A reason why the effect of liming on plant S% lessened during the experiments could be unrelated to the lime addition and rather reflect that plant N levels were decreasing, although N fertilizer

had been applied, and so as less protein was being formed less S was needed: there does appear to be evidence of decreasing plant N levels during the experiments (data not presented).

The method of lime application had no effect on the S content of PRG at the first harvest in Experiment 2B (Figure 3.25a) but had a very highly significant effect at the second (Figure 3.25b), as had occurred for Mo. However, when the results of the second harvest were studied in more detail with regard to pH the effect of method of application did not seem to be real (see page 122, for further details). That is for a given increase in pH regardless of how it was achieved, the same size and pattern of increases occurred in herbage S; extractable soil SO_4^{2-}-S levels were not investigated in Experiment 2B but it would be interesting to ascertain whether method of lime application affects soil SO_4^{2-}-S contents.

4. FERTILIZATION

Three of the experiments (3, 4 and 5) undertaken for this thesis investigated the effects of the addition of fertilizer (N, P or Cu) on the soil-plant relationships of Cu, Mo and S; the literature relating to fertilization is reviewed in Chapter I, Section 3.1d.

4.1 Lime x phosphorus application

The effect that varying rates of lime and P additions had on the soil-plant relationships of Cu, Mo and S was studied in Experiment 4 (see Chapter III, Section 2.4). Two lime additions were made to raise the soil pH to *c.* 5.0 and 6.5 so, as explained on page 136, the effect of P application and liming *per se* was not investigated rather

the modifying effect of pH on P application. In contrast to the other discussion sections the results for an element other than Cu, Mo and S - P - will be discussed briefly, mainly considering those points that can be related to the three elements of primary interest.

Phosphorus

There was no significant effect of liming on the Morgan's extractable PO_4^{3-}-P content of either soil nor was there a significant interaction between lime and phosphorus (Figure 3.37). Haynes (1982) has reviewed the effect that liming has upon the phosphate availability of acid soils and records the existence of conflicting evidence as to whether liming increases, decreases or does not affect soil extractable phosphate. In a later paper (1983), Haynes proposes that liming will increase P adsorption because of the formation of "new highly-active adsorbing surfaces in the soil" via the precipitation of amorphous polymer hydroxy-Al from exchangeable Al. These new adsorbing surfaces may also affect the behaviour of SO_4^{2-} and MoO_4^{2-} but to a lesser extent because the order of specific adsorption with regard to pH is $PO_4^{3-} > MoO_4^{2-} > SO_4^{2-}$ (Parfitt, 1978). Haynes was studying a Spodosol (approximating to U.K. podzols and some peaty podzols) and obviously soil type will significantly alter how liming affects the adsorption of PO_4^{3-} (and other anions).

It is possible that, particularly in soil 2 which contained 1050 mg Al kg^{-1} oven-dry soil there might have been an effect of liming on the soil available phosphate content had the unlimed soil (pH *c.* 3.5) been compared with the limed soil; but no such comparison was made and at the pHs studied the Al had probably precipated out.

The application of lime affected plant P concentration but there was no consistent pattern, the application of P increased herbage P content; however there was no interaction between liming and P-addition (Figure 3.43). Soil pH was not correlated with plant P concentration at any time. Haynes (1982) records that reports exist of unaltered, increased and decreased plant P contents after liming and P-additions: as for soil P content, an effect might have been noted had an unlimed and limed soil been compared.

Correlation analyses were performed between plant and soil P contents and significant correlations were found: $p < 0.05$ at the second harvest, $p < 0.001$ for initial soil concentration and herbage concentration at the first harvest for WC and PRG, and at the second harvest for WC; and $p < 0.01$ for the initial soil concentration and PRG concentration at the second harvest.

Copper

The extractable soil Cu content of soil 2 was slightly increased by liming whilst that of soil 8 was unaffected (see page 211for discussion of this). There was no interaction between lime and P-addition in either soil, but P-addition (alone) did slightly increase the Cu content of soil 8, but not that of soil 2 (Figure 3.33).

The lack of effect of P on extractable soil Cu in soil 2 is consistent with the observations of El-Kherbawy and Sanders (1984) who could find no effect of soil P-status on either extractable soil Cu concentration or the concentration of Cu^{2+} in soil soltuion. It is possible that complex Cu phosphates (e.g. $Cu_5(H_2PO_4)_2(OH)_8$ or $Cu_3(PO_4)_2$) were formed in soil 8 leading to decreased extractable soil Cu as suggested by Jurinak and Inouye (1962) and proposed by Dolar and Keeney (1971) to account for the decreases they observed in extractable soil Cu following P-addition.

By the second harvest the response of plant Cu concentration to liming was that discussed on page 213, i.e. decreased Cu contents for WC and PRG growing in both soils (Table A3.35). There was basically no interaction between lime and P-addition (only in soil 2 at the second harvest was the interaction significant, at $p < 0.05$). The effect of P addition was inconsistent and variable (soils 2 and 8 at the first harvest $p < 0.05$, soil 2 at the second harvest $p < 0.001$, and soil 8 at the second harvest non-significant) - the most common effect was slightly decreased or unaltered herbage Cu content as the amount of P applied rose (Figure 3.39).

From studying the literature (see page 32) it was expected that even although P-addition basically had no effect on extractable soil Cu contents there would have been a decrease of plant Cu content as observed by, for example, Reddy *et al.* (1981a). It is thought that decreases in herbage Cu contents with P-addition (although soil content remains unaltered) arise both because of a dilution effect and also because of P-induced changes "in the plant and its associated microorganisms or in the rhizosphere" (El-Kherbawy and Sanders, 1984). Despite plant Cu contents not being consistently decreased by P-fertilization the soil extractable P content was significantly negatively correlated to herbage Cu concentration ($p < 0.01$) for soil and herbage at the second harvest, and for initial soil P content and herbage at this harvest; and $p < 0.001$ for initial soil P content and herbage contents at the first harvest.

Molybdenum

The addition of lime increased the extractable Mo content of soil 2 but did not affect that of soil 8 (see page 215 for discussion) (Figure

3.34). There was neither a significant interaction between lime and P-addition nor an effect of P-addition; P-addition possibly increased soil Mo contents at the outset of the experiment but by the end of the experiment this effect was lost (Table A3.30).

It would be expected that PO_4^{3-} addition would increase the MoO_4^{2-} extractable from a soil because of anion exchange on soil surfaces particularly oxide surfaces (Parfitt, 1978), but there was no evidence for this in the two soils studied.

The response of plant Mo concentrations to lime and P-additions was the same as for soil Mo, i.e. liming elicited a response [increased Mo contents in WC grown on both soils and in PRG grown in soil 8 (see page 216 for discussion of this effect)], whereas P-addition did not. There was no significant interaction between lime x phosphorus additions at the first harvest although by the second harvest there was a highly significant interaction ($p < 0.01$) (Figure 3.40 and Table A3.36).

Again the lack of effect of P fertilization is in contrast to what is generally found in the literature, i.e. enhanced plant Mo uptake (e.g. Barshad, 1951; Murphy *et al.*, 1981), which is accounted for both because of decreased adsorption of Mo (Parfitt, 1978), and enhanced Mo uptake via a complex phosphomolybdate ion rather than the simple molybdate ion (Barshad, 1951). Competition for uptake, however, may occur between the uncomplexed phosphate and molybdate ions and this may affect plant Mo content.

Reddy *et al.* (1981a) observed that the Mo content of clover growing in a calcareous sand was not "greatly affected" by P-addition although in other soils plant Mo content increased, so soil type is obviously important in determining the response to P-addition.

It is possible that either the amounts of P applied were not sufficient, or the amount of soil Mo present great enough to elicit any response to P-addition. Reddy *et al.* (1981a) observed that P application had a greater enhancing influence on Mo uptake by subterranean clover when Mo fertilizer was also applied, but although the maximum P-addition was equivalent to only 67.5 kg P ha^{-1} they still obtained a response with P fertilizer alone. In order to confirm whether either of the possibilities suggested above is correct, this experiment (4) could be repeated with rates of P fertilizer over 96 kg ha^{-1}, and also with Mo fertilizer additions.

An alternative reason for the lack of response to P-addition could be that anion exchange of PO_4^{3-} and MoO_4^{2-} was not possible, for example because of inaccessibility of the oxide surfaces. However, this suggstion is not very plausible, particularly for soil 2 which gave the expected responses with regard to plant and soil Mo contents for the majority of treatments applied during the other experiments reported in this thesis.

Sulphur

The addition of lime to both soils did not initially have an effect on soil extractable SO_4^{2-}-S content; but after plant growth there was more SO_4^{2-} extractable from pots limed to *c.* 6.5 rather than 5.0, where WC had been grown but no difference was noted where PRG was grown (Table A3.31). Possible causes for the increased soil SO_4^{2-} concentrations with raised pH are discussed on page 218. The effect of P addition was analagous to that of liming, no effect initially in either soil or in either soil after PRG growth but an effect did occur in the pots in which WC was grown - a decrease (Figure 3.35). There was no lime

x P-addition interaction (at the end of the experiment) but the species x lime x P-addition was significant ($p < 0.05$).

The general lack of effect of P fertilization on soil SO_4^{2-} contents, as for soil Mo contents, was not expected since SO_4^{2-} contents, like those of MoO_4^{2-}, are generally reported to be enhanced by P-application, although to a lesser extent than MoO_4^{2-}, but for the same reason, i.e. increased competition for anion adsorption (e.g. Metson and Blakemore, 1978). The difference in effect that the two species had on soil extractable SO_4^{2-} contents with regard to both lime and P-addition could be related to their different growth rates. WC produced considerably less herbage than PRG (Figure 3.38) and hence utilized less soil SO_4^{2-}. Therefore at the end of the experiments, pots in which WC were grown would contain more SO_4^{2-} than those in which PRG had been grown, 13-78 mg SO_4^{2-}-S kg^{-1} air-dry soil for WC pots compared with 8-26 mg kg^{-1} for PRG; presumably insufficient SO_4^{2-} was desorbed to replace all that utilized.

An alternative explanation for the difference between the two species could be related to the reputation of WC as a soil acidifier (Helyar, 1976). No evidence of this was, however, found in this experiment where bulk soil pHs were measured although rhizosphere pH was possibly reduced. If WC had acidified the soil, even if only in the rhizosphere, this would have caused a reduction in soil SO_4^{2-} levels (by analogy with the effect of liming), but there was no evidence of this. Instead it was PRG growth that led to reduced soil SO_4^{2-} levels during the course of the experiment, and WC growth led to unaltered or slightly increased levels.

The addition of lime led to increased herbage S concentrations although this did not continue beyond the first harvest for PRG, the effect of lime addition on herbage S content is discussed on page 218. P-addition had a very highly significant but inconsistent effect on herbage S% (at both harvests), the basic effect was decreased plant S% as the amount of P applied increased. There was no interaction between lime and P-addition at the first harvest, i.e. at both lime levels P-addition decreased herbage S contents, but there was a highly significant interaction ($p < 0.01$) at the second, i.e. P-addition only had an effect at lime level 1. At both harvests the species x lime x P-addition interaction was significant ($p < 0.001$ at the first harvest and $p < 0.01$ at the second) (Figure 3.41).

The decrease of plant S contents following P fertilization was, as for soil SO_4^{2-} contents, the opposite of what would be expected from reading the literature (e.g. McLaren, 1976; Reddy *et al.*, 1981a). However, P-addition to one of the soils studied by Reddy did not lead to any alteration in the S content of subclover. Again no adequate explanation can be offered as to why herbage S contents did not behave as expected, a soil effect could be proposed, but since the two soils used were very different it seems improbable that it was a soil effect or, at least, that if a soil effect was the cause it would have been the same in both soils.

4.2 Nitrogen application

The discussion of the effect that N application has on the soil-plant relationships of Cu, Mo and S will be based on the results of Experiment 3 (Chapter II, Section 2.3).

Copper

There was no evidence of any effect of N fertilization on the herbage Cu concentration of PRG growing in either soil - neither a dilution effect (*cf.* Chaudhry and Loneragan, 1970; Reith *et al.*, 1984) nor an effect of the form of N fertilizer applied (*cf.* Tills and Alloway, 1981a) was observed (Figure 3.29). It had been expected that soil 8, at least, might have produced a herbage Cu response to N fertilization. Soil 8 is a peaty podzol with a low to deficient EDTA-extractable soil Cu content, and the literature contains several reports of lowered herbage Cu concentrations after N application to Cu deficient peats (e.g. DeKock and Cheshire, 1968, for wheat). Responses of plant Cu following N addition to soils which contain adequate EDTA-extractable soil Cu, such as soil 2, have also been reported (e.g. Reith *et al.*, 1984, observed increased herbage Cu concentrations after N-addition). The lack of effect of N fertilizer on herbage Cu contents for both soils in the present study illustrates how unpredictable trace-element response to fertilziation is.

The maximum amount of N applied in Experiment 3 was only 80 kg N ha^{-1} which is a moderate field dressing, and the DM production response of PRG growing in soil 2 had not yet reached its maximum and plateaued (Figure 3.28). It would be interesting to see if the application of more N, for example 150 or 200 kg N ha^{-1}, would produce a herbage Cu response, particularly since there is reported to be an association between N and Cu within the plant for translocation purposes (e.g. Loneragan *et al.*, 1980).

Much of the published work about the relationship between N fertilization and plant Cu uptake has been carried out with Cu deficient soils to which both N and Cu have been added (e.g. Hill *et al.*, 1978

and Singh and Swarup, 1982, for sands; Cheshire *et al.*, 1967, for peats). Hence, it is possible that the addition of Cu also to soils 2 and (particularly) 8 might have elicited a different response to that described above.

Singh and Swarup (1982) accounted for increased herbage Cu concentration for plants growing in a loamy sand following N ($(NH_4)_2SO_4$) and Cu application because of increased soil availability of Cu compounds due to the acidifying effect of the $(NH_4)_2SO_4$ together with greater uptake by an enlarged plant root system. However, in Experiment 3 herbage DM production increased with N application, particularly for soil 2, as presumably did root production, yet there was no corresponding increase in plant uptake, possibly because enough Cu was already being absorbed by the plants.

Molybdenum

There was no response of PRG herbage Mo concentration to N fertilization for plants grown in soil 8 but in soil 2, the soil which produced the largest growth response to N, N application (regardless of form or level of N addition) led to reduced Mo concentrations (Figure 3.30). These observations suggest that N application to soil 2 produced a dilution effect on herbage Mo content. However, since this 'dilution effect' did not continue beyond 40 kg N ha^{-1} while the growth response did, it is possible that the decrease in Mo concentration was brought about by the presence of N.

The possibility that N application reduces the herbage Mo concentration of PRG grown in soil 2 is supported by data from Experiments 1B and 4, but not by that from Experiments 2A and 5 (Table 4.2); in all experiments, other than Experiment 3, an initial application of NH_4NO_3

Table 4.2: Comparison of herbage Mo concentrations for PRG grown in soil 2 (data for PRG plants grown for a similar length of time under similar conditions to those of Experiment 3).

Experiment	pH at harvest	Days of growth	Mo concentration of PRG (mg kg^{-1} DM)	s.e.
1B	5.0	35	2.0	0.29
	4.9	60	5.4	0.72
	5.0	90	6.1	0.66
2A	5.1	40	4.0	0.57
3 No N	*5.0*	*42*	*4.3*	*0.32*
N+N	*5.0*	*42*	*3.1*	*0.45*
4	4.6	36	1.4	1.11
5	5.1	30	7.2	1.48

equivalent to 60 kg N ha^{-1} was made. The lack of complete support for this hypothesis does not rule it out as differences between the experiments (e.g. treatment differences, time of year) may account for the varying herbage Mo concentrations.

A decrease in PRG (and red clover) Mo concentration was observed by Williams and Thornton (1972) following the application of $(NH_4)_2SO_4$ and was accounted for due to the presence of the NH_4^+ ion, but no mechanism was proposed for the effect. A possible mechanism could be that the presence of NH_4^+ ions acidified the soil which reduced Mo uptake (see page 216) (*cf.* Goldberg *et al.*, 1983, where soil acidification brought about by NH_4^+ fertilization increased plant Mn uptake). Soil 2 was observed to exhibit a greater increase in plant Mo concentration after liming than soil 8, so conversely this could explain why there was a decrease in plant Mo concentration after NH_4^+ addition only in soil 2. NO_3^--N addition has been reported to increase plant need for Mo (Gupta and Lipsett, 1981), but whether this is reflected in an increased (or

decreased) plant Mo concentration will depend upon the amount of soil available Mo.

Barshad (1951) suggested that N fertilization delays Mo uptake rather than decreasing it and this could be supported by the results of Experiment 1B (see Table 4.2) where herbage Mo concentration was observed to increase with time; but the influence of the initial lime application, and N application after each harvest make the results difficult to interpret. In order to investigate this suggestion further it would be necessary to study the long term effect of N fertilization on plant Mo uptake using several different unlimed soils, despite the problems of obtaining sufficient herbage DM production for analysis. Both WC and PRG should, if possible, be used in such a study as West (1981) has reported that clover Mo concentrations are increased and PRG Mo concentrations decreased by N application.

Why N fertilization only affected the Mo concentration of PRG grown in soil 2 and not of that grown in soil 8 seems unlikely to be related to the extractable Mo contents of the unamended soils, which were 0.042 and 0.049 mg Mo kg^{-1} oven-dry soil respectively. It has already been mentioned the greater response of plant Mo content for plants grown in soil 2 rather than soil 8 is consistent with their behaviour with regard to treatments applied in other experiments (see page 194). Soil 8 had twice the total N content of soil 2 before the application of any treatments (1.00 mg N kg^{-1} oven-dry soil *cf.* 0.42 mg kg^{-1} s.e. 0.008) which may not only account for the lack of growth response (Figure 3.28), but also for why soil 8 did not produce herbage with altered Mo response following N-addition, i.e. the N already present lead to reduced herbage Mo contents.

Sulphur

The herbage S% of PRG grown in soil 8 was affected only slightly by N addition whereas that of PRG grown in soil 2 was affected markedly (Figure 3.31). The decrease observed in soil 2 was probably a dilution effect, as also noted by McLaren (1976). However, the effect did not continue beyond 40 kg N ha^{-1} with NH_4^+-N although it did for NO_3^--N despite equivalent growth responses being produced by the two forms of N fertilizer. A similar difference in response to the different forms of N fertilizer was also observed in soil 8 where differences were smaller, as were growth responses, but were relatively greater for NO_3^--N than for NH_4^+-N. The difference between NH_4^+-N and NO_3^--N is consistent with work reported by Kirkby (1978) who observed higher anion (including SO_4^{2-}) concentrations in plants supplied with NH_4^+-N rather than NO_3^-N which was accounted for by increased protein formation in NH_4^+-N fed plants; and is also consistent with the work of Blair *et al.* (1970) who explained the difference in terms of altered cation-anion balances, the explanation was also that favoured by Jarvis and Robson (1983).

The difference in the effect of N fertilizer on the two soils is analogous to the results obtained for herbage Mo concentration as would be expected since both Mo and S are present in the soil as anions and behave similarly both in terms of soil reactions and plant uptake.

4.3 Copper application

The discussion of the effect of Cu fertilizer on the soil-plant relationships of Cu, Mo and S is based on the results of Experiment 5 (Chapter III, Section 2.5).

Copper

The addition of Cu (as CuO) at a rate equivalent to 10 kg Cu ha^{-1} led to large increases in the EDTA-extractable soil Cu concentrations of both soil 2 and soil 8, particularly the latter (Table 3.5). The same quantity of CuO had been added to each soil (0.010 g pot^{-1}) as application was made on a kg ha^{-1} basis, calculated from the surface area of the pot. So, since soil 8 was less dense than soil 2 (270 g per pot *cf.* 420 g) the concentration of Cu applied was greater in soil 8 than soil 2 - 30 mg Cu kg^{-1} air-dry soil compared with 19 mg kg^{-1} and hence the concentration extracted was also greater.

The amount of EDTA-extractable Cu was greater after 50 days of plant growth than at the start of the experiment despite insoluble CuO being used as the fertilizer (CuO was added in preference to $CuSO_4$ because S was one of the three elements being studied). It therefore appears that the CuO was slowly dissolving in the soils either because the soils were quite acid (they had pHs 5.0 to 5.5) or for some other unidentified soil factor. The slow release of Cu^{2+} from the CuO could have been brought about by the plant growth, e.g. root exudation and consequent alteration of rhizosphere conditions, but as the plant species grown did not affect the extractable soil Cu content but did affect the herbage Cu concentration (Figure 3.45) this is unlikely. Further evidence that the CuO was slowly dissolving is drawn from a consideration of the work of Williams and McLaren (1982) who observed that when soils were incubated moist after Cu-addition (small quantities of readily available $CuCl_2$) the amount of EDTA-extractable Cu decreased with time.

The increase in extractable soil Cu content when Cu fertilizer was applied was reflected by increased herbage Cu concentrations for

plants grown in the soils (Figure 3.45), particularly for soil 8 which naturally had a low extractable soil Cu content. (There was no effect for WC grown in soil 2.) Increased herbage Cu concentrations in pastures after Cu fertilization have frequently been observed (e.g. Burridge *et al.*, 1983).

There was a strong correlation between the measured herbage and soil Cu contents at the second harvest ($p < 0.001$) and there were also correlations between the initial extractable soil Cu content and the herbage Cu concentrations at the first ($p < 0.001$) and second harvests ($p < 0.05$).

Molybdenum and sulphur

Cu-addition had no effect on the amount of extractable soil Mo (Table 3.9) or on the herbage Mo concentration of PRG but it did lead to raised Mo content in WC at the first harvest only (Figure 3.46). It is unlikely, however, that this latter result was real 'cause and effect' relationship although both small antagonistic (Mackay *et al.*, 1966) and small synergistic effects (Gupta and MacLeod, 1975) have been reported in the literature for the Cu x Mo interaction in plants.

There was also no effect of Cu fertilizer on extractable soil SO_4^{2-}-S concentrations (Table 3.10) or herbage S contents (Figure 3.47). MoO_4^{2-} and SO_4^{2-} are both anions which react similarly in the soil and would not be expected to be affected by the presence of high concentrations of Cu^{2+} cations.

5. SPECIES

The differences in the uptake of Cu, Mo and S between PRG and WC were investigated in Experiment 2A for soils 2, 4 and 8 (results in Chapter III, Section 2.2) and in Experiments 4 and 5 for soils 2 and 8 (results in Chapter III, Sections 2.4 and 2.5 respectively). The discussion that follows is based on a comparison of the overall mean for each of the two species growing in the two or three soils used in these experiments, regardless of other treatments applied (Tables 4.3 to 4.10). [The results from Experiment 5 for the soils to which Cu fertilizer had been added are not included in the copper section.]

Copper

WC was observed to have greater herbage Cu concentrations than PRG (Table 4.3); when all the herbage Cu concentrations for the two species in the three experiments (2A, 4 and 5) were meaned the mean Cu content was 6.64 mg Cu kg^{-1} DM for WC and 4.84 mg Cu kg^{-1} DM for PRG. This observation is consistent with the species differences in Cu concentrations noted by others (e.g. Forbes and Gelman, 1981; Wilkinson, 1984).

Possible reasons for species differences in Cu content have already been proposed in Chapter I, Section 3.1e. It is not possible to decide which is the most likely from the information collected in the three experiments. However, it is very probable that the difference is in some way related to interspecific differences in the mechanism of Cu uptake - Cu uptake in an active process (Bowen, 1969). For example, WC plants may have larger root systems than PRG and thus be better able to exploit the soil available Cu - monocots (e.g. grasses) and dicots (e.g. legumes) have different structures to their root systems,

Table 4.3: Comparison of mean herbage Cu concentrations for PRG and WC grown during Experiments 2A, 4 and 5 in soils 2, 4 and 8.

Soil	Experiment	Harvest	*Mean herbage Cu concentration (mg kg^{-1} DM)*					
			PRG			WC		
			Mean	Range	s.e.	Mean	Range	s.e.
2	2A	1	7.1	6.6 - 7.9	0.21	8.9	8.6- 9.4	0.18
		2	4.6	4.4 - 4.6	0.09	8.1	7.6- 8.5	0.09
	4	1	6.8	5.0 - 9.7	0.17	8.1	7.3- 9.5	0.14
		2	6.9	6.2 - 7.5	0.07	9.0	8.0-10.8	0.07
	[1]5	1	11.1	-	0.25	11.7	-	0.25
		2	7.5	-	0.42	13.9	-	0.42
4	2A	1	5.5	5.0 - 5.8	0.21	7.5	6.8- 7.9	0.21
		2	3.9	3.2 - 5.0	0.09	10.6	6.3-13.0	0.09
8	2A	1	2.4	2.4 - 2.5	0.21	2.3	2.0- 2.7	0.21
		2	1.9	1.7 - 2.2	0.09	1.8	1.3- 2.1	0.09
	4	1	2.2	1.8 - 2.8	0.17	2.8	2.0- 4.4	0.13
		2	2.5	2.1 - 2.7	0.07	2.1	1.7- 2.3	0.07
	5	1	2.8	-	0.25	2.7	-	0.25
		2	2.5	-	0.42	3.5	-	0.42
	$\overline{x}$		*4.84*			*6.64*		

[1] Values for Experiment 5 are the means of one treatment only - that without added Cu, so no range of values for the experiment are given.

the former have many adventitious and lateral roots while the latter have a main tap root (Russell, 1977). Alternatively, WC may have a greater rate of Cu uptake than PRG. It would have been informative to have looked at the roots of the two species to assess whether there were differences in the rooting systems; or if the difference in above ground (herbage) Cu concentrations was also present below ground in the roots, which are known to retain Cu (e.g. Jarvis, 1980).

Table 4.4: Mean EDTA-extractable soil Cu contents at the beginning and end of Experiments 4 and 5 for soils 2 and 8 in which PRG or WC was grown.

Soil	Experiment	*EDTA-extractable soil Cu (mg kg^{-1} air-dry soil)*		
		‡‡ T0	T2	
			PRG	WC
2	4	3.5	4.8	4.6
	5 †	†3.8	4.3	4.3
8	4	0.45	0.56	0.54
	5 †	†0.50	0.63	0.48

s.e. Experiment 4 T0 = 0.049, T2 = 0.028

†one value only not replicated analysis - soils without added Cu. ‡‡ see Table 4.6.

An alternative reason why WC contained more Cu than PRG could be unrelated to the uptake mechanism *per se*, but rather related to the availability of Cu from the soil: WC may have increased the amount of plant available Cu from the soil, for example by root exudation by changing the soil redox potential. If, however, Table 4.4 is studied it is seen that there was very little difference in extractable soil Cu contents after PRG or WC growth; and if there is a difference it is that soils in

which WC had been grown contains less Cu than those in which PRG had been grown - the reverse of what would have been expected. The growth of both species led to raised EDTA-extractable soil Cu contents.

It is possible that had rhizosphere soil been analysed rather than bulk soil, differences might have been observed in the extractable soil Cu contents as recorded by Linehan *et al.* (1985) and discussed on page 193. This section also draws attention to the lack of correlation between EDTA-extractable soil Cu and herbage Cu contents, although it was found for both harvests in Experiment 4 that initial soil Cu content was very highly significantly correlated ($p < 0.001$) with PRG and WC Cu concentrations (both together and separately).

Mitchell *et al.* (1957a) reported that whilst for plants grown in a soil with adequate Cu legumes will contain more Cu than grasses, when grown in a soil of low to deficient Cu grasses will contain more than legumes. The Cu contents of soil 8 is low according to the limit set by MacKenzie (1974), although no plant growth response was observed after Cu fertilizer application in Experiment 5 (Figure 3.44). Not only did the plants grown in soil 8 have the lowest herbage Cu concentrations, indicating a relationship between soil Cu status and plant Cu content, but also they exhibited the smallest differences between WC and PRG Cu concentrations and, in one instance, the second harvest of Experiment 4 WC herbage contained less Cu than PRG herbage in agreement with Mitchell *et al.* (1957a).

Mitchell (1963) accounts for the alteration in the relative herbage Cu content of grasses and legumes, with changes in soil Cu status, because there is a greater variation in the range of herbage Cu values found in legumes than in grasses. Evidence was found in Experiments 2A and 4 (a similar comparison was not possible in Experiment 5) that

WC plants do have a wider range of herbage Cu values for a given set of conditions than PRG plants, e.g. soil 2 at the second harvest of Experiment 4 produced WC plants containing 8.0 - 10.8 mg Cu kg^{-1} DM and PRG plants containing 6.2 - 7.5 mg kg^{-1} DM. But although varying ranges may account for the phenomenon described above, it does not explain it, that is why, on soils with low levels of extractable Cu, legume herbage contains less Cu than grass herbage when it has already been proposed that legumes have a greater ability (for whatever reason) than grasses to absorb Cu. It seems improbable that the *ability* of a plant of either species to absorb Cu will be modified by soil Cu status and it is therefore suggested that the result for soil 8 at the second harvest of Experiment 2 is an 'aberration' and should not be given undue weight and that the order legumes > grasses with regard to herbage Cu content is not altered by soil Cu status - this is in agreement with the findings of Beavington and Wright (1977) and Forbes and Gelman (1981), although not with Mitchell *et al.* (1957a).

Molybdenum

WC was observed to have greater herbage Mo concentrations than PRG (Table 4.5); when all herbage Mo concentrations for the two species in the three Experiments (2A, 4 and 5) were meaned the mean Mo concentration of WC herbage was 6.41 mg kg^{-1} DM and that of PRG herbage 4.64 mg kg^{-1} DM. This observation is consistent with the differences in Mo concentrations between PRG and WC noted by others, e.g. Barshad (1948) and Forbes and Gelman (1981).

Mo uptake appears to be an active process although there is no direct evidence (Moore, 1972). Therefore, as for Cu, the difference between the relative Mo contents of WC and PRG is probably related

Table 4.5: Comparison of mean herbage Mo concentrations for PRG and WC grown during Experiments 2A, 4 and 5 in soils 2, 4 and 8.

Soil	Experiment	Harvest	*Mean herbage Mo concentration (mg kg^{-1} DM)*					
			PRG			WC		
			Mean	Range	s.e.	Mean	Range	s.e.
2	2A	1	4.4	1.9 - 7.4	0.33	6.4	2.1 - 13.8	0.29
		2	5.2	2.3 - 8.4	0.28	5.7	2.6 - 11.9	0.28
	4	1	2.8	1.2 - 5.3	0.45	14.2	1.6 - 41.2	0.37
		2	5.4	3.5 - 8.4	0.43	10.7	3.0 - 19.2	0.39
	5[2]	1	7.2	7.1 + 7.2	1.05	5.9	4.1 + 7.6	0.74
		2	14.3	13.1 + 15.5	3.41	4.1	3.2 + 5.0	2.41
4	2A	1	1.9	1.8 - 2.1	0.33	2.6	1.8 - 3.7	0.33
		2	2.7	2.4 - 3.0	0.28	3.1	2.5 - 3.5	0.28
8	2A	1	2.1	1.7 - 2.6	0.33	2.8	1.1 - 5.1	0.33
		2	3.0	2.3 - 2.8	0.28	1.3	1.3 - 1.4	0.28
	4	1	2.0	1.5 - 2.6	0.45	7.9	2.7 - 14.9	0.33
		2	3.6	2.5 - 4.8	0.43	4.1	1.9 - 9.0	0.42
	5[2]	1	3.6	3.2 + 4.0	1.05	7.6	5.2 + 10.0	1.05
		2	6.8	6.6 + 6.9	3.41	13.3	3.3 + 23.3[†]	3.41
	$\bar{x}$		*4.64*			*6.41*		

[†]The 4 replicates for this value were 44.3, 41.9, 4.5 and 2.4.

[2]Mean of two treatments.

to interspecific differences in uptake mechanisms or to differences in the effect the two species have on the availability of soil Mo. e.g. via root exudates. From Table 4.6 it can be seen that although growth

Table 4.6: Mean NH_4OAc-extractable soil Mo contents at the beginning and end of Experiments 4 and 5 for soils 2 and 8 in which PRG or WC was grown.

Soil	Experiment	*NH_4OAc extractable soil Mo ($mg\ kg^{-1}$ air-dry soil)*		
		†T0	T2	
			PRG	WC
2	4	0.087	0.058	0.066
	5	0.082	0.057	0.066
8	4	0.038	0.024	0.034
	5	0.063	0.026	0.034

s.e. Experiment 4: T0 = 0.0048, T2 = 0.0020
Experiment 5: T0 = 0.0077, T2 = 0.0035

†T0, T2: days 0 and 52 for Experiment 4, days 0 and 50 for Experiment 5.

of both species caused an overall reduction in the quantity of Mo extractable from soil 2 and soil 8 the reduction was greater after PRG growth (the extractable soil Mo contents were remarkably similar in both Experiment 4 and Experiment 5). This result could indicate the WC does have the ability to increase soil Mo availability; but if the amount of Mo taken up is considered rather than the concentration in the herbage (Table 4.7) it is seen that, apart from soil 2 in Experiment 4, WC took up less Mo than PRG so the soil difference could merely reflect the lower DM production of and need for, Mo by WC. The points raised in the discussion about species differences in Cu content must also be remembered here, namely: differences in the rooting systems of legumes

and grasses both with regard to soil exploration/exploitation and the possible retention of nutrients within the roots and also that bulk soil, not rhizosphere soil, was analysed.

Table 4.7: Comparison of the mean amounts of Mo taken up by PRG and WC grown in soils 2 and 8 during Experiments 4 and 5.

Soil	Experiment	*Mean Mo uptake ($\mu g\ pot^{-1}$)*	
		PRG	WC
2	4	13.0	15.6
	5	29.4	3.8
8	4	9.6	5.7
	5	15.4	13.9

When correlations were performed for Experiment 4 between the soil extractable Mo at the outset of the experiment and the herbage Mo concentration at the two harvests the following correlations were obtained:

Harvest	Species	Significance of correlation
1	PRG + WC	*
	PRG	*
	WC	NS
2	PRG + WC	***
	PRG	**
	WC	**

No correlation was found between soil Mo content at the second harvest and the herbage Mo contents of the two species considered together at this harvest. This indicates that trying to relate herbage Mo content to NH_4OAc-extractable soil Mo content is not straightforward, or totally valid.

Although less Mo, in absolute terms, was taken up by legumes than grasses there was more in relative (concentration) terms, which could possibly be related to the ability of legumes to fix nitrogen. Mo is an essential component of nitrogenase, the enzyme which is produced by the N_2-fixing bacterium *Rhizobium* which lives in legume root nodules and it is known that legumes require more Mo than grasses, in order to supply this need (Mengel and Kirkby, 1982). If, in order to supply this requirement legumes have a more efficient Mo uptake mechanism than grasses and all the 'extra' Mo taken up is not utilized in N_2 fixation there will be a higher concentration of Mo in the plant - probably in both shoots and roots (root analysis for Mo would confirm or refute this idea).

Sulphur

WC was observed to have lower herbage S concentrations than PRG when grown in soils 2 or 4 but generally greater S concentrations when grown in soil 8 (Table 4.8). When all herbage S% for the two species in the three Experiments (2A, 4 and 5) were meaned the means were 0.187% DM for PRG and 0.223% for WC grown in soil 8, and 0.295% for PRG compared to 0.216% for WC grown in soils 2 and 4. If all three soils were taken together the means were 0.231% for PRG and 0.198% for WC. There is no consistent difference reported in the literature with regard to the relative S% of legumes and grasses; legumes < grasses (Metson, 1979), legumes $\overset{\text{grasses}}{\simeq}$ (McLaren, 1976) and legumes > grasses (Gilbert and Robson, 1984).

Sulphur is taken up by plants as SO_4^{2-} during an active process (Bouma, 1975) so there would be expected to be good correlation between extractable soil SO_4^{2-} content and herbage S concentration. Correlations

Table 4.8: Comparison of mean herbage S concentrations for PRG and WC grown during Experiments 2A, 4 and 5 in soils 2, 4 and 8.

Soil	Experiment	Harvest	*Mean herbage S concentration (% DM)*					
			PRG			WC		
			Mean	Range	s.e.	Mean	Range	s.e.
2	2A	1	0.26	0.23 - 0.31	0.005	0.21	0.20 - 0.22	0.004
		2	0.25	0.22 - 0.26	0.003	0.15	0.14 - 0.18	0.003
	4	1	0.41	0.31 - 0.56	0.008	0.25	0.20 - 0.30	0.006
		2	0.31	0.28 - 0.34	0.006	0.21	0.18 - 0.26	0.005
	5[1]	1	0.44	0.44 + 0.44	0.008	0.28	0.28 + 0.28	0.006
		2	0.27	0.26 + 0.28	0.004	0.27	0.27 + 0.27	0.003
4	2A	1	0.25	0.22 - 0.27	0.005	0.21	0.21 - 0.23	0.005
		2	0.17	0.15 - 0.20	0.003	0.15	0.13 - 0.16	0.003
8	2A	1	0.18	0.14 - 0.23	0.005	0.20	0.17 - 0.23	0.005
		2	0.08	0.08 - 0.09	0.003	0.10	0.09 - 0.12	0.003
	4	1	0.28	0.21 - 0.39	0.008	0.27	0.20 - 0.30	0.006
		2	0.12	0.10 - 0.14	0.006	0.23	0.14 - 0.29	0.006
	5[1]	1	0.35	0.34 + 0.36	0.008	0.29	0.28 + 0.30	0.008
		2	0.11	0.11 + 0.12	0.004	0.25	0.24 + 0.25	0.004
	$\bar{x}$		*0.231*			*0.198*		
	$\bar{x}$ *(soils 2 and 4 only)*		*0.295*			*0.216*		
	$\bar{x}$ *(soil 8 only)*		*0.187*			*0.223*		

[1]Mean of two treatments.

were performed between plant and soil S contents for Experiment 4 and there was some degree of correlation between the soil SO_4^{2-} content at the outset of the experiment and herbage S% when both species were considered together and for PRG, but not WC alone:

Harvest	Species	Significance of correlation
1	PRG + WC	*
	PRG	**
	WC	NS
2	PRG + WC	*
	PRG	*
	WC	NS

There was a very highly significant ($p < 0.001$) correlation between soil SO_4^{2-}-S and herbage S contents at the second harvest for PRG and WC considered together (the two species were not analysed separately). However, if the soil SO_4^{2-}-S values at the second harvest are studied (Table 4.9) it is seen that both for soil 2 and for soil 8 the soil in which PRG had been grown contained less extractable SO_4^{2-} than the soil in which WC had been grown; although only in soil 8 did PRG herbage contain less S. A similar pattern of results was also observed in the soil from Experiment 5. In both experiemnts and for both soils the extractable SO_4^{2-}-S content was relatively unaffected by WC growth but was considerably decreased by PRG growth, and if the amount of S taken up per pot is calculated (Table 4.10) WC is observed to consistently take up less S than PRG, regardless of soil.

It is therefore proposed that in all three soils studied (2, 4 and 8), PRG had a higher S concentration than WC and that the apparent reversal of this in soil 8 was not 'real' but rather related to the greater S uptake of PRG. If the mean herbage S concentrations obtained on soil 8 are studied in more detail (Table 4.8) it is found that at the first

Table 4.9: Mean PO_4^{3-} extractable soil $SO_4^{2-}S$ contents at the beginning and end of Experiments 4 and 5 for soils 2 and 8 in which PRG or WC was grown.

Soil	Experiment	*PO_4^{3-} extractable soil $SO_4^{2-}S$ (mg kg^{-1} air-dry soil)*		
		†T0	T2	
			PRG	WC
2	4	57	21	57
	5	52	27	50
8	4	47	10	42
	5	37	10	25

s.e. Experiment 4: T0 = 2.8, T2 = 1.0
Experiment 5: T0 = 2.3, T2 = 0.7

†T0, T2: days 0 and 52 for Experiment 4
days 0 and 50 for Experiment 5.

Table 4.10: Comparison of the mean amounts of S taken up by PRG and WC growing in soils 2 and 8, during Experiments 4 and 5.

Soil	Experiment	*Mean S uptake (mg pot^{-1})*	
		PRG	WC
2	4	13.1	2.9
	5	7.9	2.2
8	4	8.6	3.1
	5	6.6	3.4

harvest of Experiments 2A and 4 PRG and WC had approximately the same S content, and for Experiment 5 PRG contained more S than WC, but at the second harvests PRG contained considerably less S than WC. Whitehead (1972) states that typical grass S concentrations are 0.20-0.45% but at the second harvest of all three experiments the PRG S% was below this range - 0.08% in Experiment 2A, 0.12% in Experiment 4 and 0.11% in Experiment 5. It is therefore suggested that soil 8 has a lower ability to supply a plant's S need, and that whereas for WC, with its lower uptake (Table 4.10) the soil can supply sufficient SO_4^{2-} this is not possible for PRG which has a greater S uptake reflected by the reduced soil SO_4^{2-} concentrations after PRG growth (Table 4.9). Hence, after a period of growth in soil 8, grasses contain less S than legumes.

The lower ability of soil 8 to supply SO_4^{2-}-S to plants does not appear to be related to soil SO_4^{2-}-S content as before the application of any treatments the SO_4^{2-}-S contents of soils 2, 4 and 8 were 30, 16 and 18 mg SO_4^{2-}-S kg^{-1} oven-dry soil respectively. The most obvious difference between the three soils is in their OM contents and possibly the association between S and OM restricts its availability to plants. That is in soil 8 the rate of S uptake by grass was greater than the rate of mineralization of OM and consequent release of SO_4^{2-}, whereas for the other two more mineral soils mineralization of OM was not so important to supply plants with SO_4^{2-} as more was available from soil clays and oxides.

6. "TIME TRENDS"

The effect that season has on plant mineral content was reviewed in Chapter I, Section 3.2. It is not possible from a series of pot experiments such as was carried out for this thesis to properly investigate the effect of season - this can only be studied in the field where the various factors contributing to the seasonal effect [e.g. day-length, temperature (ambient and soil), stage of maturity of the plants and soil drainage status] are unaltered. It is, however, possible to observe 'time-trends' from the pot experiments, since most of the six experiments had two harvests (Table 2.1). Also, since the experiments were carried out progressively over an eighteen month period, it is possible to observe if there was an effect of the time of year ('season') at which the experiments were performed on soil and herbage Cu, Mo and S concentrations and, particularly, herbage DM production. Only the results of soils 2 and 8 in which PRG was grown will be discussed as these were the two soils and one species used in all the experiments.

Dry matter production

Of all the variables assessed in the experiments the one which would have been most affected by any seasonal effects would have been herbage DM production; and it can be seen from Table 4.11 that there was an effect of 'season' on DM production.

The mean daily DM productions will reflect the effects of the different treatments imposed in the experiments, so in order to study the effect of 'season' the ranges will be considered - in particular the maximum production. DM production is seen to be greatest in the summer experiment - number 4 - and least in the winter experiment - number 1B - the other experiments which were performed in the spring and autumn gave intermediate production.

Table 4.11: Daily herbage DM production of PRG grown in soils 2 and 8 during the six experiments.

Experiment No.	Season	Harvest	PRG herbage DM production (g pot^{-1} day^{-1})			
			Soil 2		Soil 8	
			Mean	Range	Mean	Range
1B	Winter	1	0.018	0.002 - 0.026	0.025	0.002 - 0.037
		2	0.038	0.064 - 0.051	0.044	0.002 - 0.081
		3	0.035	0.009 - 0.049	0.023	0.004 - 0.041
2B	Winter/ spring	1	0.041	0.007 - 0.060	0.056	0.016 - 0.060
		2	0.051	0.044 - 0.061	0.084	0.042 - 0.063
3	Spring/summer	1	0.038	0.014 - 0.060	0.064	0.054 - 0.072
4	Summer	1	0.062	0.048 - 0.075	0.068	0.064 - 0.084
		2	0.084	0.068 - 0.097	0.089	0.070 - 0.108
5	Autumn	1	0.034	0.033 - 0.034	0.047	0.045 - 0.047
		2	0.063	0.063 - 0.064	0.077	0.076 - 0.078
6B	Spring	1	0.044	0.008 - 0.081	0.070	0.031 - 0.119

There was no consistent pattern of production during the course of the experiments as this was dependent upon the imposed treatments, frequency of harvesting and health of the plants.

Copper, molybdenum and sulphur

Mean values for the PRG herbage Cu, Mo and S, and extractable soil Cu, Mo and SO_4^{2-}-S concentrations (where measured) are presented in Tables 4.12 and 4.13. It is difficult to discern when the tables are studied whether there were any consistent trends in nutrient concentrations with time during the experiments because, relatively few values are presented (particularly for soil concentrations) and each experiment was designed to study different soil treatments, so the results are not strictly comparable. The latter problem may be partially overcome by considering the ranges of values rather than the mean values. Therefore suggestions about time trends in the patterns of behaviour of Cu, Mo and S are tentative.

(a) Copper

Soil - there was no consistent alteration in mean extractable soil Cu concentrations during the experiments.

Herbage - there was a small indication that mean herbage Cu concentrations decreased over the course of an experiment.

(b) Molybdenum

Soil - the extractable Mo concentration of soil 8 decreased during the experiments as did that of soil 2 in Experiments 4 and 5, but the extractable Mo concentration of soil 2 tended to increase with time in Experiments 1B and 6B.

Table 4.12: Mean herbage Cu, Mo and S concentrations for PRG grown in soils 2 and 8 during the six experiments.

Experiment No.	Harvest	Days of PRG growth	*Herbage Cu concentration (mg kg^{-1} DM)*				
			Soil 2		Soil 8		
			Mean	Range	Mean	Range	s.e.
1B	1	35	10.6	9.7 - 11.5	2.6	2.3 - 3.0	*0.22*
	2	25	8.4	7.7 - 9.3	2.8	2.2 - 3.4	*0.10*
	3	30	8.9	8.0 - 11.0	2.9	1.7 - 3.9	*0.09*
2B	1	40	8.0	6.6 - 11.4	2.1	1.7 - 2.5	*0.17*
	2	30	5.1	4.4 - 8.0	1.6	1.2 - 2.2	*0.08*
3	1	42	5.3	4.8 - 5.5	1.8	1.6 - 1.8	*0.04*
4	1	36	6.8	5.0 - 9.7	2.2	1.8 - 2.8	*0.17*
	2	16	6.9	6.2 - 7.5	2.5	2.1 - 2.7	*0.07*
5†	1	30	13.2	11.1 - 15.3	9.7	2.8 - 16.5	*0.18*
	2	20	9.3	7.5 - 11.2	5.7	2.5 - 8.8	*0.30*
6B	1	36	9.1	6.3 - 11.9	2.2	1.6 - 2.9	*0.10*

Experiment No.	Harvest	Days of PRG growth	*Herbage Mo concentration (mg kg^{-1} DM)*				
			Soil 2		Soil 8		
			Mean	Range	Mean	Range	s.e.
1B	1	35	2.9	1.3 - 6.0	0.94	0.6 - 1.5	*0.11*
	2	25	4.1	0.4 - 6.9	1.7	0.2 - 2.9	*0.31*
	3	30	5.8	1.2 - 10.2	4.0	1.2 - 5.4	*0.25*
2B	1	40	4.5	1.9 - 7.4	1.8	1.5 - 2.6	*0.21*
	2	30	5.8	2.3 - 9.4	2.3	1.0 - 3.8	*0.18*
3	1	42	2.9	2.1 - 4.3	2.2	1.8 - 2.6	*0.16*
4	1	36	2.8	1.2 - 5.3	2.0	1.5 - 2.6	*0.45*
	2	16	5.4	3.5 - 8.4	3.6	2.5 - 4.8	*0.43*
5	1	30	7.2	7.1 - 7.2	3.6	3.2 - 4.0	*1.05*
	2	20	14.3	13.1 - 15.5	6.8	6.6 - 6.9	*3.41*
6B	1	36	2.8	2.0 - 3.8	1.1	0.7 - 1.4	*0.08*

Experiment No.	Harvest	Days of PRG growth	*Herbage S concentration (% DM)*				
			Soil 2		Soil 8		
			Mean	Range	Mean	Range	s.e.
1B	1	35	0.34	0.28 - 0.39	0.32	0.29 - 0.43	*0.005*
	2	25	0.28	0.35 - 0.30	0.19	0.10 - 0.39	*0.004*
	3	30	0.28	0.25 - 0.32	0.16	0.10 - 0.38	*0.004*
2B	1	40	0.28	0.23 - 0.36	0.20	0.14 - 0.38	*0.005*
	2	30	0.23	0.18 - 0.26	0.09	0.06 - 0.16	*0.003*
3	1	42	0.32	0.23 - 0.38	0.14	0.12 - 0.15	*0.003*
4	1	36	0.41	0.31 - 0.56	0.28	0.21 - 0.39	*0.008*
	2	16	0.31	0.28 - 0.34	0.12	0.10 - 0.14	*0.006*
5	1	30	0.44	0.44 - 0.44	0.35	0.34 - 0.36	*0.008*
	2	20	0.27	0.26 - 0.28	0.11	0.11 - 0.12	*0.004*
6B	1	36	0.27	0.21 - 0.31	0.21	0.11 - 0.28	*0.002*

† Cu fertilizer addition was an experiment treatment.

Table 4.13: Mean soil extractable Cu, Mo and SO_4^{2-}-S concentrations for soils 2 and 8 in which PRG was grown during experiments 1B, 4, 5 and 6B.

Experiment No.	Days of PRG growth	*Extractable Cu concentration (mg kg^{-1} air-dry soil)*				
		Soil 2		Soil 8		
		Mean	Range	Mean	Range	s.e.
1B	0	4.2	3.9 - 4.6	0.60	0.58 - 0.62	*0.023*
	35	4.6	4.2 - 4.8	0.61	0.52 - 0.68	*0.025*
	60	4.7	4.4 - 4.9	0.63	0.51 - 0.72	*0.024*
	90	4.0	3.6 - 4.5	0.49	0.41 - 0.57	*0.027*
4	0	3.5	3.2 - 3.9	0.45	0.40 - 0.50	*0.049*
	52	4.8	4.6 - 5.0	0.55	0.44 - 0.66	*0.031/0.006*†
5[2]	0	7.3	3.8 - 10.9	4.8	0.5 - 9.2	*0.54*
	50	10.6	4.3 - 16.8	9.5	0.6 - 18.3	*0.34*
6B	0	3.6	1.9 - 6.8	0.33	0.24 - 0.46	*0.040*
	36	3.0	1.8 - 5.3	0.27	0.21 - 0.34	*0.023*

Experiment No.	Days of PRG growth	*Extractable Mo concentration (mg kg^{-1} air-dry soil)*				
		Soil 2		Soil 8		
		Mean	Range	Mean	Range	s.e.
1B	0	0.077	0.033 - 0.172	0.050	0.045 - 0.061	*0.0047*
	35	0.085	0.045 - 0.217	0.048	0.033 - 0.080	*0.0021*
	60	0.089	0.045 - 0.217	0.034	0.027 - 0.049	*0.0021*
	90	0.101	0.052 - 0.219	0.048	0.038 - 0.079	*0.0069*
4	0	0.087	0.043 - 0.133	0.038	0.018 - 0.047	*0.0048*
	52	0.058	0.045 - 0.073	0.024	0.019 - 0.031	*0.0020*
5	0	0.082	0.080 - 0.085	0.063	0.063 - 0.064	*0.0077*
	50	0.057	0.054 - 0.061	0.026	0.024 - 0.027	*0.0035*
6B	0	0.056	0.046 - 0.067	0.042	0.028 - 0.063	*0.0014*
	36	0.088	0.055 - 0.145	0.028	0.019 - 0.044	*0.0018*

Experiment No.	Days of PRG growth	*Extractable SO_4^{2-}-S concentration (mg kg^{-1} air-dry soil)*				
		Soil 2		Soil 8		
		Mean	Range	Mean	Range	s.e.
1B	0	49	33 - 77	31	14 - 59	*1.3*
	35	54	31 - 103	40	12 - 114	*1.7*
	60	46	26 - 96	31	8 - 112	*1.7*
	90	43	16 - 97	35	8 - 131	*1.4*
4	0	57	49 - 67	47	39 - 59	*2.8*
	52	21	17 - 26	10	8 - 13	*1.0*
5	0	52	50 - 54	37	33 - 40	*2.3*
	50	27	26 - 28	10	9 - 11	*0.7*
6B	0	49	37 - 60	48	27 - 78	*1.0*
	36	32	17 - 53	21	10 - 47	*0.8*

† s.e. for soil 2/soil 8

[2] Cu fertilizer addition was an experimental treatment.

Herbage - there was a consistent pattern of increasing herbage Mo concentrations with time during the experiments despite the lack of a similar pattern for soil extractable Mo contents. This is consistent with the generally reported view that as season advances and plants mature herbage Mo contents increase (e.g. Davies and Crawshaw, 1978), i.e. although harvests were taken at about 30 day intervals the plant roots were maturing and growing, and hence exploiting a larger volume of soil and were able to take up more Mo.

(c) Sulphur

Soil - the extractable SO_4^{2-}-S contents of both soils decreased with time during the experiments, this is consistent with the observations of Tsuji and Goh (1979).

Herbage - herbage S concentrations decreased during the course of the experiments as less soil SO_4^{2-}-S was available for uptake.

7. HERBAGE COPPER AVAILABILITY TO GRAZING SHEEP

The importance of the relative concentrations of Cu, Mo and S in herbage grazed by sheep in determining whether or not that herbage is likely to give rise to an induced copper deficiency in the animals has been discussed in Chapter I, Section 1. The equation derived by Suttle (1983b) to calculate the percentage of the ingested Cu that is absorbable by grazing sheep was also discussed in that section. The equation is:

$$\text{Cu absorption (\%)} = 5.71 - 1.279S - 2.785 \ln Mo + 0.227 Mo \times S$$

where S and Mo are the herbage concentrations of sulphur and molybdenum in g kg^{-1} DM and mg kg^{-1} DM respectively.

There are two major limitations that apply to the above equation which have prevented calculation of percentage Cu absorptions (A_{Cu}) for some of the treatment combinations in the six experiments presented in this thesis, namely: the equation is not applicable where herbage S concentrations are >4 g kg^{-1} DM and/or herbage Mo concentrations >6 mg kg^{-1} DM. Values which could not be calculated are indicated by a - in Tables 4.14 to 4.23. A further restriction is that the equation is applicable only to 'summer herbage' - the herbage used to derive the equation was collected during June-July from a heterogenous permanent pasture (Suttle, pers. comm.).

The application of the equation to data collected in glasshouse pot experiments conducted throughout the year is therefore tenuous, particularly for WC; but it provides a method of integrating the measured herbage variables of Cu, Mo and S concentrations, and of determining the effect of different improvement processes. Therefore, the A_{Cu} and T_{Cu} values which follow, and the interpretation of them, must be regarded as a guide only to what might occur for herbage collected under similar conditions in the field. More importantly, the sheep's grazing behaviour and proportions of improved and indigenous pasture to which the animal has access will have significant influences on the availability of Cu from the grazed herbage.

Another problem connected with the use of A_{Cu} and T_{Cu} values is: what are the boundary values between sufficient, marginal and insufficient copper for a grazing sheep? The factors which influence the susceptibility of a grazing sheep to induced Cu deficiency were discussed in Chapter I, Section 4.1 and it was noted that many different factors were involved. From a consideration of published values of the minimum Cu requirement for sheep (ARC, 1980) and of absorbable

herbage copper concentrations that have been observed to lead to induced Cu deficiencies in growing lambs (Whitelaw *et al.*, 1979, 1983), it is probable that the threshold value for T_{Cu}s that give rise to negative Cu balances in Blackface sheep lies in the range 0.15 - 0.20 mg Cu kg^{-1} DM: but this is not certain and it is undoubtedly better to consider the relative rather than the absolute values for A_{Cu} and T_{Cu} in the context of the work presented in this thesis.

(a) Soils

In Chapter IV, Section 1, the nine soils studied were categorized into very high, high, moderate or low risk soil series with regard to the probability of herbage growing in the soils being likely to be low in available Cu for grazing animals. The categorization used was that presented in the SAC/SARI (1982) study group report, 'Trace element deficiencies in ruminants', and was carried out on two different bases, one with regard to the probable herbage Cu concentration and the other with regard to the probable herbage Mo concentration:

Classification based on:

Herbage Cu concentration		Herbage Mo concentration	
Risk status	*Soil*	*Risk status*	*Soil*
		Very high	1
High	3,5,6,9	High	4
Moderate	2,7,8	Moderate	8
Low	1,4	Low	2,3,5,6,7,9

If the classification of the soils by the two different systems are considered together, it is seen that only one soil, number 8, is placed in the same class in both systems and that all the rest are in diametrically opposite classes.

The lack of accurate applicability of the SAC/SARI classifications to herbage Cu or Mo concentrations has already been discussed (pages 191 and 195), and the situation is no better when the availabilities of herbage copper to grazing sheep are considered. If Tables 4.14 and 4.15 are studied it is possible to derive the following rankings for the herbage A_{Cu} and T_{Cu} values (values meaned over three harvests):

A_{Cu}	Lime 0	6 > 5 > 7 > 8 > 2 > 4 > 9 > 1 > 3
	Lime 2	7 > 9 > 6 > 3 > 5 > 4 > 1 > 8 > 2
	Lime 4	8 > 6 > 7 > 4 > 9 > 5 > 3 > 1 > 2
T_{Cu}	Lime 0	2 > 6 > 5 > 1 > 4 > 9 > 7 > 3 > 8
	Lime 2	6 > 9 > 1 > 3 = 5 = 7 > 4 > 8 > 2
	Lime 4	6 > 7 > 1 > 5 > 8 > 9 > 2 = 4 > 3

It can be seen that neither do these orders reflect the SAC/SARI classifications, nor do they give a particularly consistent pattern - either between lime levels or with respect to the two ways of expressing Cu availability; although soil 6 is the soil most frequently producing herbage with copper availabilities near the top of the range observed and soil 2 is that most consistently producing herbage towards the bottom of the range.

(b) Lime

If, however, the relative decreases in copper availability brought about by liming are considered (lime level 0 *cf.* lime level 4), reasonably similar orders with regard to the magnitude of the decrease are obtained for A_{Cu} and T_{Cu} values (mean of three harvests):

A_{Cu}	2 > 5 > 9 > 1 > 6 > 7 > 4 > 3 > 8
T_{Cu}	2 > 5 > 4 > 6 > 1 > 9 > 3 > 7 > 8

largest decrease ⟷ *smallest decrease*

Table 4.14: % Availability of herbage Cu (A_{Cu}) to sheep from PRG grown in soils 1 to 9 at three lime levels (calculated from Suttle, 1983b).

Soil	*Availability of Cu (% herbage Cu)*								
	Unlimed			Lime level 2			Lime level 4		
	†T1	T2	T3	T1	T2	T3	T1	T2	T3
1	2.6	3.2	2.1	1.8	2.2	1.5	0.7	1.0	0.6
2	2.2	6.5	2.5	0.5	-	0.4	0.4	0.2	-
3	3.4	2.7	0.8	4.0	3.0	0.9	0.9	0.5	1.1
4	4.9	3.4	2.6	3.2	1.8	1.5	-	1.4	1.5
5	6.5	6.2	2.6	3.2	2.4	1.6	0.7	1.1	0.9
6	4.8	6.7	4.3	4.1	2.9	2.7	-	2.5	1.6
7	6.0	5.3	2.9	3.9	4.2	2.8	1.4	2.7	2.0
8	3.8	4.2	4.4	2.3	1.5	1.4	3.4	5.0	0.5
9	3.3	4.7	2.4	3.7	4.5	2.3	0.7	1.3	0.8

s.e.: T1 = 0.55, T2 = 0.59, T2 = 0.33

Table 4.15: Availability of herbage Cu (T_{Cu}) (mg kg^{-1} DM) to sheep from PRG growing in soils 1 to 9 at three lime levels (calculated after Suttle, 1983b).

Soil	*Availability of Cu (mg kg^{-1} DM)*								
	Unlimed			Lime level 2			Lime level 4		
	†T1	T2	T3	T1	T2	T3	T1	T2	T3
1	0.33	0.30	0.18	0.19	0.17	0.12	0.08	0.07	0.03
2	0.24	0.82	0.28	0.04	-	0.04	0.05	0.03	-
3	0.23	0.15	0.08	0.21	0.17	0.07	0.04	0.01	0.05
4	0.27	0.22	0.20	0.21	0.09	0.10	-	0.04	0.04
5	0.43	0.41	0.21	0.17	0.16	0.13	0.06	0.06	0.05
6	0.35	0.39	0.36	0.38	0.22	0.21	-	0.10	0.05
7	0.20	0.18	0.17	0.15	0.16	0.15	0.06	0.09	0.06
8	0.09	0.06	0.11	0.07	0.03	0.04	0.10	-	0.01
9	0.18	0.28	0.16	0.19	0.27	0.16	0.04	0.06	0.05

s.e.: T1 = 0.022, T2 = 0.029, T3 = 0.022

†T1, T2, T3: first, second and third harvests - days 30, 65, 90.

Soils 2 and 5 produced the largest decline in herbage Cu availability and soil 8 the smallest, on average. If, however, the results at the individual harvests are considered, it is found that soil 7 was the soil most consistently showing the smallest decreases. Soils 3 and 8 appear lower than soil 7 in the ranking because these are the only soils which gave increased Cu availabilities with liming (lime level 0 *cf.* lime level 4) - soil 8 at the first and second harvests and soil 3 at the final harvest. For soil 8, lime level 4 had been considerably overlimed and if Cu availabilities at lime level 0 are compared to those at lime level 3 (Tables 4.16 and 4.17) they are seen to decrease; soil 3 was the only soil in the study which came from a previously improved site.

The range of A_{Cu} values found in the soils was from 0.2 to 6.7% and the corresponding range of T_{Cu} values was 0.01 to *0.43 mg Cu kg^{-1} DM. All the available Cu concentrations at lime levels 3 and 4 were <0.10 mg Cu kg^{-1} DM; which would very probably give rise to Cu nutrition problems in sheep, while for the unlimed soils (except for soil 3 at the final harvest, and soil 8 - see above) all Cu concentrations were >0.15 mg kg^{-1} DM and most were >0.20 mg kg^{-1}, a concentration which would almost certainly not give rise to problems of Cu nutrition. That is, whereas the unlimed soils would not give rise to induced Cu deficiency in sheep the same soils when limed to *c.* pH 7 - 7.5 (lime level 4) will, since there is at least a 100% difference between the herbage Cu availabilities - in other situations, for example, lime level 2 in this experiment, the distinction is not so easy. The application of lime at level 2, raising pH to *c.* 5.0, gave (omitting soils 2 and 8) Cu availabilities ranging from 0.07 to 0.38 mg kg^{-1} DM and whilst some of the PRG plants would

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*Excepting plants grown on unlimed soil 2 at the second harvest which had 0.82 mg Cu available kg^{-1} DM.

Table 4.16: % Availability of herbage Cu (A_{Cu}) to sheep from PRG grown in soils 2, 4 and 8 at five lime levels (calculated from Suttle, 1983b).

Soil	*Availability of Cu (% herbage Cu)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	†T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	2.2	6.5	2.5	3.0	1.8	1.3	0.7	0.7	0.4	0.9	1.0	-	0.4	-	-
4	4.9	3.4	2.6	2.8	2.2	2.2	2.8	1.8	1.5	-	1.5	1.5	-	2.1	1.5
8	3.6	5.8	4.4	4.0	2.6	1.4	2.0	2.3	1.5	2.2	2.2	1.9	3.4	5.0	0.5

s.e.: T1 = 0.73, T2 = 0.62, T3 = 0.27

Table 4.17: Availability of herbage Cu (T_{Cu}) (mg kg^{-1} DM) to sheep from PRG growing in soils 2, 4 and 8 at three lime levels (calculated after Suttle, 1983b).

Soil	*Availability of Cu (mg kg^{-1} DM)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	†T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	0.24	0.83	0.28	0.26	0.13	0.11	0.07	0.06	0.04	0.10	0.09	-	0.05		-
4	0.27	0.21	0.20	0.22	0.16	0.17	0.20	0.11	0.10	-	0.09	0.09	-	0.07	0.05
8	0.09	0.14	0.11	0.12	0.09	0.05	0.06	0.07	0.04	0.05	0.06	0.05	0.10	-	0.01

s.e.: T1 = 0.030, T2 = 0.013, T3 = 0.017

†T1, T2, T3: first, second and third harvests - days 30, 65 and 90.

have been capable of providing adequate Cu for sheep, other plants would not.

Although soils 2 and 8 were at the opposite ends of the scale of response (see above), they both had particularly low Cu availabilities (T_{Cu}s). Whereas for soil 2 the situation was caused because lime addition dramatically increased herbage Mo contents, for soil 8 the unlimed soil naturally had a very low Cu content (*c.* 0.6 mg Cu kg^{-1} oven-dry soil) and hence low T_{Cu} values.

At the first harvest of Experiment 2A it was also observed that liming decreased the availability of Cu to grazing sheep (A_{Cu} and T_{Cu}) both from PRG and, to an apparently greater extent, from WC (Tables 4.18 and 4.19). At the second harvest of this experiment it was not possible to calculate enough A_{Cu} values for soil 2 for the effect of liming to be determined. But in soils 4 and 8 liming at this harvest apparently had no effect on the A_{Cu} from PRG whilst decreasing that of WC grown in soil 8 and increasing that of WC grown in soil 4. If T_{Cu}s rather than A_{Cu}s are considered, decreases with liming apparently occurred for WC grown in both soil 4 and soil 8 and for PRG grown in soil 4, but there was no effect for PRG in soil 8 (again no comparisons were possible for soil 2). Therefore the problem of decreasing predicted Cu availabilities to grazing sheep from herbage grown in limed soils is confirmed.

However, if the T_{Cu} values are studied more closely it is seen that soil 8 never produced herbage, of either species, containing >0.08 mg available Cu kg^{-1} DM - a level which would certainly produce a negative Cu balance in sheep. Similarly, PRG grown in soils 2 and 4 would have not adequately supplied the Cu requirements of a grazing sheep and WC could have only satisfied the need at the lowest lime level (both harvests) and the intermediate level (at the second harvest).

Table 4.18: % Availability of herbage Cu (A_{Cu}) to sheep from PRG and WC grown in soils 2, 4 and 8 at three levels of lime and, for PRG, two methods of lime application (calculated from Suttle, 1983b).

Soil	Harvest[†]	*Availability of Cu (% of herbage Cu)*								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	2.2	1.4	-	2.0	1.0	0.7	0.9	0.6	0.6
	2	2.2	2.1	-	1.9	1.0	-	1.1	-	1.0
4	1	2.3	1.8	1.1	2.2	2.1	1.7	2.3	1.6	1.7
	2	1.6	1.7	2.5	1.7	1.4	1.9	3.0	2.6	2.7
8	1	3.8	2.2	1.0	3.1	2.5	1.5	3.2	2.3	1.6
	2	4.4	4.2	3.8	2.1	2.3	2.8	3.9	4.0	4.2

s.e. Part A (Lime A): T1 = 0.21, T2 = 0.31
Part B (PRG): T1 = 0.21, T2 = 0.25

Table 4.19: Availability of herbage Cu (T_{Cu}) (mg kg^{-1} DM) to sheep from PRG and WC grown in soils 2, 4 and 8 at three levels of lime and, for PRG, two methods of lime application (calculated after Suttle, 1983b).

Soil	Harvest[†]	*Availability of Cu (mg kg^{-1} DM)*								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	0.19	0.13	-	0.13	0.07	0.06	0.07	0.05	0.06
	2	0.17	0.18	-	0.09	0.05	-	0.05	-	0.08
4	1	0.18	0.13	0.08	0.12	0.10	0.09	0.12	0.07	0.09
	2	0.22	0.21	0.16	0.09	0.04	0.07	0.15	0.10	0.10
8	1	0.07	0.05	0.03	0.08	0.06	0.04	0.06	0.04	0.03
	2	0.08	0.08	0.05	0.05	0.05	0.05	0.06	0.05	0.05

s.e. Part A (Lime A): T1 = 0.013, T2 = 0.017
Part B (PRG): T1 = 0.007, T2 = 0.008

[1]Harvest 1,2: Days 40, 70

[2]A1, A2, A3: Lime applied throughout the soil at levels 1, 2 and 3
S1, S2, S3: Lime applied to surface (top 30%) of soil at levels 1, 2 and 3.

The method by which lime was applied to the soil (Experiment 2B - Tables 4.18 and 4.19) did seem to affect the availabilities of Cu to sheep but did not alter the general pattern of decreasing availability with lime application. In soil 2 where lime was applied to the surface layer of soil, herbage A_{Cu} values were lower than where lime was applied throughout the soil, but for soils 4 and 8 surface applied lime increased the A_{Cu} values. If T_{Cu} values were considered (together with pH differences - see page 109), the values were less affected by the method of lime application and those which were affected did not give a consistent pattern of response. All the T_{Cu} values calculated for PRG were <0.15 mg available Cu kg^{-1} DM (most <0.10 mg kg^{-1} DM) regardless of lime application method and would have led to negative Cu balances in grazing sheep.

(c) Fertilization

Lime x phosphorus

No values for the availability of Cu to grazing sheep are presented for Experiment 3, where N fertilization was studied, or Experiment 5, where Cu fertilization was studied. This is because for the former experiment there was no effect of N addition on Cu availabilities, and for the latter virtually no values were calculable because of high herbage Mo and/or S concentrations. The effect of P fertilization was investigated in Experiment 4 and, with the exception of soil 2 at lime level 2, A_{Cu} and T_{Cu} values could be calculated for most treatments - Tables 4.20 and 4.21.

There appeared to be no effect of P addition to soil 2 at lime level 1 on herbage A_{Cu} values or generally on T_{Cu} values, although the P increment 48 to 96 kg P ha^{-1} did increase the availability of Cu

Table 4.20: % Availability of herbage Cu (A_{Cu}) for PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels (calculated from Suttle, 1983b).

Soil	Lime level	*Availability of Cu (% herbage)*											
		WC						PRG					
		[1]P1		P2		P3		P1		P2		P3	
		[2]T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
2	1	2.3	1.2	1.7	1.1	2.2	1.4	-	0.7	1.6	0.9	2.1	0.9
	2	-	-	-	-	-	-	-	-	-	0.8	0.8	0.9
8	1	1.7	1.4	1.0	1.2	1.7	2.8	1.9	1.7	2.2	1.5	2.6	1.5
	2	-	1.3	-	0.8	-	1.4	0.8	1.4	1.1	2.3	1.8	2.0

s.e.: T1 = 0.344, T2 = 0.175

Table 4.21: Availability of herbage Cu (T_{Cu}) (mg kg^{-1} DM) for PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels (calculated after Suttle, 1983b).

Soil	Lime level	*Availability of Cu (mg kg^{-1} DM)*											
		WC						PRG					
		[1]P1		P2		P3		P1		P2		P3	
		[2]T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
2	1	0.17	0.13	0.16	0.10	0.18	0.13	-	0.05	0.10	0.06	0.10	0.06
	2	-	-	-	-	-	-	-	-	-	0.06	0.04	-
8	1	0.06	0.03	0.02	0.02	0.03	0.06	0.04	0.04	0.05	0.04	0.05	0.04
	2	-	0.03	-	0.02	-	0.03	0.02	0.02	0.03	0.06	0.04	0.05

s.e.: T1 = 0.0071, T2 = 0.0087

[1]P1, P2, P3 = phosphorus levels 1, 2 and 3 equivalent to 24, 48 and 96 kg P ha^{-1}.
[2]T1, T2 = first and second harvests - days 36 and 52.

from WC (no comparisons were possible at lime level 2). P application to soil 8 did not alter the A_{Cu} or T_{Cu} values for PRG grown at lime level 1 but increased them at lime level 2 (P-level 1 *cf.* P-level 3). Although for WC grown at lime level 1 in soil 8 there was no effect of P addition on its A_{Cu} at the first harvest its T_{Cu} decreased, and at the second harvest both A_{Cu} and T_{Cu} values were increased. For WC grown at lime level 2 in soil 8 it was not possible to ascertain the effect of P-addition, but at the second harvest P-addition did not affect A_{Cu} or T_{Cu} values. Therefore, the general effect of P-fertilization on herbage Cu availabilities seems to have been inconsistent varying with soil, lime-level and species grown; but none of the plants grown in either soil could have supplied enough Cu to prevent a negative Cu balance arising in grazing sheep.

(d) "Drainage"/soil water status

It is believed that poor drainage and waterlogging reduces the availability of herbage Cu to sheep (e.g. SAC/SARI, 1982). But it can be seen from Tables 4.22 and 4.23 that the A_{Cu} and T_{Cu} values for herbage growing in soils maintained at 100% FC were higher than those grown in soils at 60% FC. Also, the A_{Cu} and T_{Cu} values were higher for plants which had been grown in soils that had been incubated moist or waterlogged than for soils which had been stored dry; basically because the incubated soils supported herbage containing less Mo than the dry pre-treated soil (Chapter III, Section 2.6).

Table 4.22: % Availability of herbage Cu (A_{Cu}) for PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months (calculated from Suttle, 1983b).

Soil	Watering regime (% FC)	*Availability of Cu (% herbage Cu)*		
		†Dry	Moist	Waterlogged
2	60	0.72	1.20	1.19
	100	0.91	1.53	1.50
8	60	2.84	2.88	2.71
	100	3.48	4.01	5.03

s.e.: 0.144

†Soil pre-treatment

Table 4.23: Availability of herbage Cu (T_{Cu}) (mg kg^{-1} DM) for PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months (calculated after Suttle, 1983b).

Soil	Watering regime (% FC)	*Availability of Cu (mg kg^{-1} DM)*		
		†Dry	Moist	Waterlogged
2	60	0.072	0.105	0.141
	100	0.057	0.105	0.163
8	60	0.049	0.054	0.091
	100	0.059	0.067	0.130

s.e.: 0.0088

†Soil pre-treatment

8. CONCLUDING DISCUSSION AND SUGGESTIONS FOR FUTURE WORK

The basic objectives of this thesis, given in Chapter I, Section 5, were: to increase the body of knowledge that exists about the soil-plant relationships of Cu, Mo and S with particular reference to "the effect that the various processes undertaken during hill improvement" have, and to provide information that might possibly help towards providing "a basis for selecting hill sites for improvement, and improvement techniques least likely to give rise to induced Cu deficiency in sheep".

In Chapter I, a table (Table 1.6) was presented which summarized "the reported influences of various factors upon the availability of copper, molybdenum and sulphur to plants". From the results presented in this thesis it is possible to produce a similar table (4.24) for comparison (Table 1.6 is presented again for reference).

Table 1.6: Summary table of the reported influences of various factors upon the availability of copper, molybdenum and sulphur to plants.

Factor		Element Cu	Mo	S
pH		↓↑	↑	↑
OM		↓	?	↑
Poor drainage		↓↑	↑	↓?
Season		√	√	√
Other nutrients:	N	↑↓	↓?	↓
	P	↓(↑)	↑	↑
	K	↓?	↓?	↓?
	others	√	√	√
Microorganisms		Mycorrhiza	Rhizosphere	Mineralization

↑ - increase
↓ - decrease
↑↓ - increases and decreases reported
? - literature is inconclusive
√ - an interaction occurs

NB This table is a generalization, specific interactions will depend on soil type, initial soil nutrient status, plant species, plant.

Table 4.24: Summary table of the observed effects (generalized) of various factors upon extractable soil Cu, Mo and SO_4^{2-}-S and herbage Cu, Mo and S concentrations.

Factor	*Element*						A_{Cu}/T_{Cu}
	Cu		Mo		S		
	Soil	Plant	Soil	Plant	Soil	Plant	
pH	↑	↓	↑	↑	↑	↑	↓
Drainage 1†	$↓_{2,=8}$	-	$↑_{2,=8}$	-	↓	-	-
2	↑	↓	$↑_{2,=8}$	↓	↓	↑↓	↑
3	=	=	=	=	↓	↓	↑
N	-	=	-	↓	-	↓	?
P	$=_2, ↓_8$	(↓)	(↑)	=	=	↓	↑↓
Cu	↑	↑	=	=	=	=	↑?
Species	-	WC>PRG	-	WC>PRG	-	WC<PRG	↑↓ $WC>PRG_2$ $WC=PRG_8$

† 1 effect of waterlogging the soil.

2 effect of previously waterlogged soil compared to soil previously stored dry or moist.

3 effect of maintaining soil at 100% FC throughout plant growth compared to 60% FC.

↑ increase
↓ decrease
= no effect
↑↓ increases and decreases
- no comparison possible - data not collected
? insufficient data to allow a comparison
2,8 soil 2, soil 8
( ) small effect

If Tables 1.6 and 4.24 are compared, it can be seen both that some of the 'gaps' in the former have been filled, e.g. the effect of N fertilization on plant Mo concentrations and also that some of the observations conflict with previously published work, e.g. the effect of drainage

on Mo concentrations for soil 8, and the effect of P fertilization on soil and herbage Mo and S concentrations. The first objective given above has, therefore, been fulfilled.

The use of Suttle's (1983b) equation to integrate the data collected for herbage Cu, Mo and S concentrations has already been discussed together with the restrictions to its use (page 253). Nevertheless, calculated A_{Cu} and T_{Cu} values are the only objective means available to assess the impact that the various soil treatments investigated would have on the Cu balance of a sheep grazing herbage growing in the soil; and hence the only means available to take steps towards fulfilling the second objective, given above.

When the A_{Cu} and T_{Cu} values were calculated (see Section 7 of this chapter) it was found that in the majority of cases - 80% - the herbage would have supplied <0.15 mg available Cu kg^{-1} DM the threshold value suggested on page 255 for the development of negative Cu balances in grazing Blackface sheep. The figure of 80% is made up of: 60% of the herbage samples grown which had T_{Cu} values <0.15 mg Cu kg^{-1} DM and 20% of the samples for which it was not possible to apply the equation because the herbage Mo and/or S concentration was too high and hence, by implication, herbage Cu availability would have been low. The *c.* 20% of cases in which the plants would have supplied >0.15 mg available Cu kg^{-1} DM nearly all came from Experiment 1A at lime levels 0 and 2 - i.e. the natural unameliorated soils or when the soil was limed to *c.* pH 5.5. It thus appears that all of the improvement practices investigated reduced the availability of herbage Cu to grazing sheep; however, it has been observed that A_{Cu} and T_{Cu} values for herbage growing in a given soil in a pot experiment are lower than those for herbage growing in the same soil in the field under similar conditions (C.C. Evans, pers. comm.).

It is relevant to discuss here the 'peculiarities' of pot experiments and why they are used; as this is relevant not only to the Cu availability indices but also to all the measurements made in the six experiments.

Pot experiments are frequently used instead of field experiments as they are a relatively cheap and easy way to investigate the relative effects of many different treatments on soil and herbage nutrient levels; but any conclusions which are drawn from them require confirmation from field trials.

The removal of a soil from the field for use in a pot experiment immediately alters the soil - its natural structure and drainage status are lost. The soil is then transported to a laboratory where it undergoes further changes - stones are sieved out and the natural ped structure disrupted further, the soil is mixed to form a homogenous medium so any OM present at the surface is distributed throughout the soil; and the soil may then be dried which considerably alters the soil flora and fauna, killing them or causing them to enter a state of dormancy.

Next (maybe after being stored dry for several months) the soil is mixed intimately with fertilizers and other amendments and is packed into a pot - which provides only a restricted volume and depth for plant root development. The soil is then rewetted thus bringing about a burst of microbial activity and nutrient release (the 'Birch' effect - see page 21), and the pots are placed in a glasshouse (or growth room) where temperature and day-length are artificially controlled. The soil temperature is thus probably higher than it would be in the field and day-length possibly longer (although the quality of the light will be poorer); so when the sown seeds germinate (with a higher percentage germination than would be obtained in the field) not only are the plants in an ideal

environment - warm, light, well-supplied with nutrients and water (although evaporation may lead to concentration of the nutrients at the soil surface) - but also they do not face competition from other species.

The factor which appeared to exert the strongest influences over the relative Cu, Mo and S concentrations of the soils and of the plants growing in the soils, and which modified the effect of the applied treatment is the one factor which cannot be altered if it is decided to improve - the *soil type*. See Table 4.24 for examples of the different behaviour of soils 2 and 8. As soil type did play such a predominant role it was therefore fortunate that soils 2 and 8, the two soils used in all six experiments, were so different, with contrasted reactions to many of the treatments. This was opportune since the results of the soil and plant analyses were not obtained until after the programme of experimental work had been completed. Particular problems were encountered with analysis for soil and herbage Mo because of the difficulty of measuring this element together with, for the herbage, the small size of the samples.

If the two soils which were chosen for use in all the experiments had reacted similarly to the applied treatments, although interpretation of the experimental results might have been more straightforward, this would have obscured the great influence that soil type does have on the soil-plant relationships of Cu, Mo and S and would possibly have implied that the subject is more easily understood than is the case.

The processes undertaken during hill land improvement were reviewed on pages 18 and 19. Once it has been decided to improve a site among the first operations to be performed is site clearance. Sites can be cleared in one of several ways (see page 18) and the method chosen may itself affect the subsequent soil-plant relationships of Cu,

Mo and S. For example, if herbicides are used to kill the existing vegetation what effect do they have? Do they increase or decrease or not alter the relative availabilities of Cu, Mo and S to the plant species that will be sown? And if they do affect the relative availability of Cu, Mo and S, how do they mediate the effect? And is there a type of herbicide that is less likely to, or more likely to, exacerbate the problem of induced Cu deficiency in livestock subsequently grazing the site? If the site is cleared by ploughing what is the effect of the incorporation of the OM into the soil? Also, what is the interaction between OM and poor drainage? OM is known to strongly influence the behaviour in the soil of all three elements of interest (see pages 19 - 22), but it was not possible in the time available to study its effect (quantity and nature) in this project.

After the site has been cleared lime and fertilizers are applied; does the method of application matter? For example, in Experiment 2B lime was applied either to all the soil in the plant pot or to just the top 30% and differences were noted only really for herbage Mo concentrations (Figure 3.23), but what is the effect in the field of a true surface dressing of lime (scattered on the soil surface) as opposed to lime incorporated into the soil? If the lime is applied as a split dressing (a common practice) rather than as a single dressing (by either method) what effect does this have, does it modify the soil and plant responses to liming? Also, does the form of fertilizer matter? In Experiment 3, different forms of N produced little difference in plant response in the two soils studied, but what about in other soils? Or for the other major nutrients? If Cu fertilizer is applied, is the form of Cu added important?

It was only possible to study PRG and WC in the experiments undertaken in this thesis, but what are the relative Cu, Mo and S contents

of the indigenous hill species? For example, if a species, e.g. a herb, or a variety of PRG or WC were found that had high Cu availabilities whether because of a high Cu concentration (see Jones and Moseley, 1984), or low Mo and S concentration, would it be possible to incorporate the species or variety into the reseeded pasture, either as a constituent of the seed mixture sown or as discrete 'mosaic' patches?

Once the hill has been improved how does the presence of the animals affect the availability of Cu to themselves from the pasture? For example, if plants with high Cu availabilities were supplied on a mosaic what would be the grazing pattern - would such plants be selected for, or against? Would the benefit derived from animals not developing negative Cu balances outweigh the loss of production that might occur because of the poorer quality of the 'mosaic' plants? Does herbage growing in patches which have been urinated on contain more Mo than that in other areas? (Urine raises soil pH, and Mo availability is increased by increasing pH.)

Most of the suggestions for investigation outlined above are most suitable for investigation in the field, as would be a study of the effect of season on the soil-plant relationships of Cu, Mo and S. However, since so little is known there are many smaller scale pot experiments that could be set up either based on the ideas above or on those that follow. Probably the most important aspect to investigate if a full understanding of how the treatment applied in the experiment mediated their effects is the forms, and total concentrations of, the three elements in the available soil pool. Throughout the discussion it was frequently found that since the interactions involved were so complicated probably the only way it would be possible to begin to understand what is happening would be to know the relative proportions of the different forms of Cu, Mo and S in the soil pool.

Another recurring theme in the discussion was the lack of knowledge of what was occurring in the rhizosphere rather than the bulk soil and this aspect needs further investigation. Also, the related subject of plant roots needs investigating - how are the rooting characteristics affected by increased pH and the other treatments applied? Are the roots of different species affected differently? Do the roots produce exudates affecting the availability of Cu, Mo and S? And, if so, how is the production of the exudates affected by the various treatments applied? Is Cu retained in the roots as is reported in the literature (see page 10)? If it is, to what extent are Mo or S also retained in the root? What effect does rooting depth have on plant uptake of Cu, Mo and S?

It can now be appreciated that although the work presented in this thesis has increased what is known about the soil-plant relationships of Cu, Mo and S in hill pastures, there is still much work that needs to be carried out in order to gain a full understanding of these relationships. Such an understanding is required to make it possible to assess the suitability of a hill site for improvement with regard to the potential effects on the Cu balances of grazing sheep.

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APPENDICES

APPENDIX I

Details of the fertilizer amendments

applied in the Experiments

Appendix 1.1: Fertilizer amendments in Experiment 1.

(a) Lime Additions

Table A1.1: Amounts of lime applied to the nine soils in Experiment 1 at lime levels, 1, 2, 3 and 4†.

Soil No.	*Amount of lime applied*							
	mg kg^{-1} air-dry soil [3]				g pot^{-1}			
	[2]L1	L2	L3	L4	L1	L2	L3	L4
1		4.3		22.7		2.04		10.67
2	2.0	5.0	10.0	26.7	0.84	2.10	4.20	11.21
3		0.3		8.0		0.15		3.52
4	1.7	4.7	10.0	26.7	0.70	1.96	4.20	11.21
5		4.0		26.0		1.60		10.40
6		6.7		28.0		1.47		6.16
7		5.3		30.7		1.97		11.36
8	4.3	10.0	19.3	66.7	1.16	2.70	5.21	18.01
9		1.0		13.3		0.42		5.59

† Lime level 0 - no lime applied, soils at natural pH (range 3.0 - 4.9).
[2] Lime levels 1, 2, 3 and 4 calculated to give soil pHs of 4.25, 5.0 5.75 and 6.5, respectively.
[3] All soils air-dry except No. 6, which was stored moist at 4°C.

(b) NPK Additions

g pot^{-1} (kg ha^{-1} equivalent)

at start of experiment:

0.169 g solid KH_2PO_4 (P - 48, K - 60)
0.409 g $Ca(NO_3)_2$ in solution (N - 60)

after first harvest:

0.205 g $Ca(NO_3)_2$ in solution (N - 30)

after second harvest:

0.307 g $Ca(NO_3)_2$ in solution (N - 45)

Appendix 1.2: Fertilizer amendments in Experiment 2.

(a) Lime Additions

Table A1.2: Amount of lime applied in Experiment 2 at the three lime levels.

Soil No.	*Amount of lime applied*					
	mg lime kg^{-1} air-dry soil			g pot^{-1}		
	†L1	L2	L3	L1	L2	L3
2	2.3	5.4	11.8	0.94	2.27	4.96
4	3.8	7.2	12.0	1.58	3.04	5.04
8	5.0	11.8	21.0	1.35	3.17	5.67

†L1, L2, L3: lime levels 1, 2, 3 which were calculated to give soil pHs of 4.5, 5.5 and 6.5, respectively.

(b) NPK Additions

g pot^{-1} (kg ha^{-1} equivalent)

at start of experiment:

0.169 g solid KH_2PO_4 (P - 48, K - 60
0.409 g $Ca(NO_3)_2$ in solution (N - 60)

after first harvest:

0.205 g $Ca(NO_3)_2$ in solution (N - 30)

Appendix 1.3: Fertilizer amendments in Experiment 3.

(a) Nitrogen Additions

Table A1.3: Amount of nitrogen fertilizer applied in Experiment 3 at nitrogen levels 1 and 2†.

Nitrogen form	Amount of nitrogen applied (g pot^{-1})	
	Level 1	Level 2
NO_3^- ($Ca(NO_3)_2$)	0.273	0.547
NH_4^+ (NH_4HCO_3)	0.183	0.366
Urea	0.069	0.139

†For N-level 0 no nitrogen addition was made
N-level 1 is equivalent to 40 kg N ha^{-1}
N-level 2 is equivalent to 80 kg N ha^{-1}.

(b) Other Fertilizers Applied

(g pot^{-1})

Lime:

Soil 2	2.25 g
Soil 8	3.18 g

P and K at start of experiment:

0.169 g solid KH_2PO_4

(P - 48 kg ha^{-1}, K - 60 kg ha^{-1})

Appendix 1.4: Fertilizer amendments in Experiment 4.

(a) Lime Additions

Table A1.4: Amount of lime applied in Experiment 4 for the two lime levels†.

Soil No.	*Amount of lime applied*			
	mg g^{-1} air-dry soil		g pot^{-1}	
	Level 1	Level 2	Level 1	Level 2
2	3.7	10.7	1.56	1.50
8	8.7	22.2	2.36	6.00

†Lime levels 1 and 2 were calculated to give soil pHs of 5.0 and 6.5, respectively.

(b) NPK Additions

Table A1.5: Amounts of NPK fertilizer applied at the start of Experiment 4 for the three phosphorus levels†.

Chemical (form)		Amount of fertilizer applied (g pot^{-1})		
		P-level 1	P-level 2	P-level 3
$CaH_4(PO_4)_2$	(solid)			0.160
KH_2PO_4	(solid)	0.085	0.169	
KNO_3	(solid)	0.063		
NH_4NO_3	(in solution)	0.114	0.139	0.139

†Phosphorus levels 1, 2 and 3 which correspond to 24, 48 and 96 kg P ha^{-1}, respectively.

After the first harvest:

0.093 g NH_4NO_3 in solution was applied to each pot (40 kg N ha^{-1}).

Appendix 1.5: Fertilizer amendments in Experiment 5.

(a) *Copper Additions* (mg pot^{-1})

At Cu level 1, 10.0 mg CuO was applied (10 kg Cu ha^{-1})

(b) *Lime Additions* (g pot^{-1})

Soil 2 - 2.25 g
Soil 8 - 3.18 g

(c) *NPK Additions* (g pot^{-1}) (kg ha^{-1} equivalent)

at start of experiment:

0.169 g	KH_2PO_4	(solid)	(P - 48, K - 60)
0.409 g	$Ca(NO_3)_2$	(in solution)	(N - 60)

after first harvest:

0.273 g	$Ca(NO_3)_2$	(in solution)	(N - 40)

Appendix 1.6: Fertilizer amendments in Experiment 6.

(a) Lime Additions

Table A1.6: Amount of lime applied in Experiment 6†.

Soil No.	Wt soil taken in Part A (g)	*Amount of lime applied*		
		mg g^{-1} air-dry soil	Part A g $bucket^{-1}$	Part B g pot^{-1} †
2	6250	8.0	50	3.36
8	4000	17.5	70	4.72

†Lime added to dry pre-treatment at outset of plant growth experiment.

(b) NPK Additions

(g pot^{-1}) (kg ha^{-1} equivalent)

at start of Experiment 6B (pot experiment):

0.169 g KH_2PO_4 (in solution) (P - 48, K - 60)

0.409 g $Ca(NO_3)_2$ (in solution) (N - 60)

APPENDIX II

Additional characters to describe

the twenty-four soils initially screened

Site No.[1]	Site location	National grid reference	Soil association	Soil series	Soil drainage status	Dry matter (%)	Organic C (%)	Extractable nutrients (mg kg^{-1} oven dry soil) Al	PO_4^{3-}-P
1A	Carron Valley Stirlingshire	NS 706825	Darleith	Myres	Poor/very poor	98.0	3.7	940	9.1
1B	Carron Valley Stirlingshire	NS 728805	Darleith	Dunlop	Imperfect	97.5	5.9	340	1.8
2a	Gillrigg Dumfriesshire	NY 064875	Yarrow	Yarrow	Free	98.0	12.4	120	14.0
2b	Parkgate Dumfriesshire	NY 005885	Ettrick	Kedslie	Imperfect	98.5	2.9	45	9.2
2c	Gillrigg Dumfriesshire	NY 056879	*Alluvial/Peat*		Poor	97.5	5.1	95	26.0
2d	Parkgate Dumfriesshire	NY 002876	*Alluvial*			97.5	12.1	155	8.2
3A	Glensaugh-Birnie Kincardineshire	NO 667790	Strichen	Fungarth	Free	96.5	13.0	800	5.2
3B	Glensaugh-Birnie Kincardineshire	NO 666788	Strichen	Fungarth	Free	98.5	4.5	240	5.3
4A	Glensaugh-West Finella Kincardineshire	NO 677777	Strathfinella	Garrold	Free (below iron pan)	98.0	9.7	310	4.7
4B	Glensaugh-West Finella Kincardineshire	NO 673776	Strathfinella	Strathfinella	Free	98.0	6.6	470	3.9
5A	Hartwood Lanarkshire	NS 836599	Rowanhill	Rowanhill	Poor	98.0	6.8	270	1.3
5B	Hartwood Lanarkshire	NS 838597	Rowanhill	Rowanhill	Poor	97.5	7.1	90	2.2
6A	House O'Muir Midlothian	NT 218628	Bemersyde	Bemersyde	Free	96.0	12.6	860	7.8
6B	House O'Muir Midlothian	NT 223623	Sourhope	Sourhope	Free	98.0	6.3	320	5.3
7A[2]	Lephinmore Argyllshire	NR 012925	*Peat*		Poor/very poor	93.0	40.0	45	51.0
7B	Lephinmore Argyllshire	NR 014925	*Peat*		Poor/very poor	92.5	45.0	<1	71.0
8A	Redesdale Northumberland	NY 831965	[3]		Very poor	97.5	8.0	190	3.8
8B	Redesdale Northumberland	NY 832964			Very poor	98.5	3.5	30	10.0
9A	Sourhope I Roxburghshire	NT 842216	Sourhope	Sourhope	Free/ intermediate	97.5	9.5	210	6.3
9B	Sourhope I Roxburghshire	NT 843207	Sourhope	*Skeletal soil*		97.0	9.7	105	44.0
10A	Sourhope II Roxburghshire	NT 864212	Sourhope	Cowie	Free with peaty top	96.5	23.0	400	14.0
10B	Sourhope II Roxburghshire	NT 858209	Sourhope	Atton	Poor	95.5	27.0	100	62.0
11A	South Slipperfield Peeblesshire	NT 123499	Bemersyde	Bemersyde	Free	99.0	3.7	190	3.2
11B	South Slipperfield Peeblesshire	NT 127498	Eckford	Eckford	Free	98.0	7.6	410	3.9
	Standard error					*0.09*	*0.61*	*13.6*	*1.27*

[1] A - indigenously vegetated site
B - improved site
All samples from location 2 (Dumfriesshire) were from improved areas.

[2] The analyses for the peat were performed on air-dry samples (as for all the soils); and are not strictly comparable to the other values presented.

[3] Soil type 'T' as defined in 'The Soils of Redesdale Experimental Husbandry Farm, Northumberland', MAFF.

APPENDIX III

Primary data (treatment means) for the Experiments

NB for an explanation of ¨ see page 65.

Appendix 3.1: Data from Experiment 1.

Table A3.1: pHs of soils 1 to 9 in which PRG was grown at three lime levels.

Soil	pH											
	Unlimed				Lime level 2				Lime level 4			
	[1]T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
1	4.0	4.1	4.1	4.1	5.0	4.9	5.0	4.9	7.3	7.2	7.3"	7.3
2	3.8	3.7	3.6	3.7	5.4	5.0	4.9	5.0	7.5	7.4	7.4	7.5
3	4.9	4.9	4.9	5.0	5.1	5.0	5.1	5.1	7.2	7.1	7.2"	7.2
4	3.3	3.4	3.4	3.5	4.8	4.7	4.6	4.7	7.4	7.4	7.3	7.4
5	3.7	4.0	4.0	3.9	5.0"	4.8	4.9"	4.7	7.2	7.3	7.3	7.3
6	3.0	3.3	3.3	3.4	4.8	4.7	4.6"	4.6	7.2	7.4"	7.2	7.2
7	3.5	3.6	3.6	3.7"	5.0"	5.0	4.8	4.7	7.5	7.4	7.5	7.4"
8	3.4	3.5	3.4	3.5	5.2	4.9	4.8	4.8	7.8	7.6	7.4	7.5
9	4.4	4.4	4.4	4.4	4.7	4.7	4.8	4.7	7.1	7.0	7.0"	7.1

s.e.: T0 = 0.02; T1 = 0.03; T2 = 0.02; T3 = 0.02

[1]T0, 1, 2, 3: 0, 35, 60, 90 days - start of experiment, first, second and third harvests.

Table A3.2: pHs of soils 2, 4 and 8 in which PRG was grown at five lime levels.

Soil	pH																			
	Unlimed				Lime level 1				Lime level 2				Lime level 3				Lime level 4			
	[1]T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
2	3.8	3.7	3.6	3.7	4.4	4.3	4.2	4.3	5.4	5.0	4.9	5.0	6.4	6.0	5.9	5.9	7.5	7.4	7.4	7.5
4	3.3	3.4	3.4	3.5	3.9	3.9"	3.8	4.0	4.8	4.7	4.6	4.7	6.2	5.9	5.7	5.7	7.4	7.4	7.3	7.4
8	3.5	3.5	3.4	3.5	4.3	4.1	4.0	4.2	5.2	4.9	4.8	4.8	6.4	6.0"	5.7	5.8	7.8	7.6	7.4	7.5

s.e.: T0 = 0.01; T1 = 0.02; T2 = 0.02; T3 = 0.02

[1]see Table A3.1

Table A3.3: Extractable Cu contents of soils 1 to 9 in which PRG was grown for 90 days at three lime levels.

Soil	*Extractable soil Cu (mg kg^{-1} air-dry soil)*					
	Unlimed		Lime level 2		Lime level 4	
	[1] T0	T3	T0	T3	T0	T3
1	1.8	1.7	1.6	1.4	2.0	2.1"
2	4.6	4.0	3.9	3.6	4.1	4.5
3	0.8	0.7	0.8"	0.6	0.9	0.9
4	2.0	1.6	1.9	1.4	2.1	2.1
5	1.9	1.5	1.6	1.4	1.8	2.0
6	12.0	12.3	12.0	13.1	10.6	11.1
7	1.1	0.9	1.0	0.8	1.1	1.2
8	0.6	0.5	0.6	0.5	0.6	0.4
9	1.0	0.8"	1.0	0.8	1.2"	1.2

s.e.: T0 = 0.11; T3 = 0.13

[1] see Table A3.1

Table A3.4: Extractable Cu contents of soils 2, 4 and 8 in which PRG was grown at five lime levels.

Soil	*Extractable soil Cu (mg kg^{-1} air-dry soil)*																			
	Unlimed				Lime level 1				Lime level 2				Lime level 3				Lime level 4			
	[1]T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
2	4.6	4.7	4.7	4.0	4.1	4.4	4.7	3.9	3.9	4.2	4.4	3.6	4.6	4.7	4.8	4.1	4.1	4.8	4.9	4.5
4	2.0	1.9	2.0	1.6	1.8	1.9	2.0	1.6	1.9	1.9	1.9	1.4	2.0	1.9	2.0	1.6	2.1	2.3	2.4	2.1
8	0.6	0.7	0.7	0.6"	0.6	0.6"	0.7	0.5	0.6	0.6	0.6"	0.5	0.6	0.6	0.6	0.5	0.6	0.5	0.5	0.4

s.e.: T0 = 0.05; T1 = 0.06; T2 = 0.05; T3 = 0.06

[1] see Table A3.1

Table A3.5: Extractable Mo contents of soils 1 to 9 in which PRG was grown for 90 days at three lime levels.

Soil	*Extractable soil Mo (mg kg^{-1} air-dry soil)*					
	Unlimed		Lime level 2		Lime level 3	
	[1]T0	T3	T0	T3	T0	T3
1	0.011	0.019	0.013	0.024	0.033	0.037
2	0.033	0.052	0.052	0.083	0.170	0.220"
3	0.027	0.030	0.029	0.033	0.035	0.035
4	0.029	0.050	0.022	0.027"	0.027	0.042
5	0.024	0.032	0.039	0.039	0.068	0.092
6	0.086	0.096	0.082	0.096	0.089	0.130
7	0.033	0.053	0.030	0.052	0.052	0.068
8	0.046	0.044	0.045	0.038	0.061	0.079
9	0.027	0.043	0.024	0.042	0.043	0.047

s.e.: T0 = 0.0078; T3 = 0.0121

[1]see Table A3.1

Table A3.6: Extractable Mo contents of soils 2, 4 and 8 in which PRG was grown at five lime levels.

Soil	*Extractable soil Mo (mg kg^{-1} air-dry soil)*											
	Unlimed				Lime level 1				Lime level 2			
	[1]T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
2	0.033	0.045	0.045	0.052	0.042	0.050"	0.049	0.061	0.052	0.053	0.052	0.083
4	0.029	0.031	0.035	0.050	0.025	0.022	0.021	0.042"	0.022	0.020	0.016	0.032"
8	0.046	0.039	0.033"	0.044	0.045	0.042"	0.033	0.040	0.045	0.033	0.027	0.038

Soil	*Extractable soil Mo (mg kg^{-1} air-dry soil)*							
	Lime level 3				Lime level 4			
	T0	T1	T2	T3	T0	T1	T2	T3
2	0.086	0.063"	0.082	0.089	0.170	0.220"	0.210"	0.220"
4	0.018	0.018	0.020	0.031"	0.027	0.028	0.026	0.042
8	0.052	0.040	0.030	0.041	0.061	0.077"	0.050"	0.079

s.e.: T0 = 0.0104; T1 = 0.0047; T2 = 0.0047; T3 = 0.0155

[1] see Table A3.1

Table A3.7: Extractable soil SO_4^{2-}-S contents of soils 1 to 9 in which PRG was grown for 90 days at three lime levels.

Soil	*Extractable soil SO_4^{2-}-S (mg kg^{-1} air-dry soil)*					
	Unlimed		Lime level 2		Lime level 4	
	[1]T0	T3	T0	T3	T0	T3
1	40	28	48	30	89	83
2	33	30	43	24	79**	97
3	14	8	14	6	20	7
4	17	13	22	12	57	42
5	49	44	33	46	67	155**
6	27	23	28	15	54	62
7	21	11	20	13	21**	14**
8	25	7	27	11	52**	135
9	19	11	19	10	45**	32

s.e.: T0 = 2.7; T3 = 3.4

[1]see Table A3.1

Table A3.8: Extractable soil SO_4^{2-}-S contents of soils 2, 4 and 8 in which PRG was grown at five lime levels.

Soil	*Extractable soil SO_4^{2-}-S (mg kg^{-1} air-dry soil)*																			
	Unlimed				Lime level 1				Lime level 2				Lime level 3				Lime level 4			
	[1]T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
2	33	35	35	30	37	31	26	16	43	39	28	24	56	62	45	46	77**	100**	96	97
4	17	20	15	13	19	12	11	6	22	28	18	16	33	28	18	16	57	65	45	43
8	25	17	8	9**	28	11	10	10	27	20**	12**	11	14**	37	11**	15	59**	115**	115**	130**

s.e.: T0 = 3.0; T1 = 3.9; T2 = 3.8; T3 = 3.0

[1] see Table A3.1

Table A3.9: Dry matter production of PRG grown in soils 1 to 9 at three lime levels.

Soil	*Dry matter production (g^{-1} pot)*								
	Unlimed			Lime level 2			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3
1	0.47	0.90	0.99	0.73	1.04	1.13	0.89	1.60	1.54
2	0.07	0.16	0.26	0.85	1.07	1.18	0.32	1.22	1.58"
3	0.95	0.84	0.55	0.98	0.92	0.59	0.83	1.21	1.04
4	0.14	0.54	0.86	1.19	1.21	1.14	0.62	1.59	1.25
5	0.25	0.45	0.57	0.87	1.33	1.20	0.89"	1.42	1.35
6	0.71	1.01	0.54	1.33	1.18	0.83	0.81"	1.40	0.81
7	1.20	1.25	0.74	1.53	1.80	1.31	0.36	1.88"	2.03
8	0.56	0.97	0.47	1.29	1.44	0.92	0.07	0.04	0.12
9	0.98	1.29	0.87	1.04	1.42	1.03	0.74"	1.60"	1.63"

s.e.: T0 = 0.039; T2 = 0.053; T3 = 0.042

[1] see Table A3.1

Table A3.10: Dry matter production of PRG grown in soils 2, 4 and 8 at five lime levels.

Soil	*Dry matter production (g^{-1} pot)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	0.08	0.16	0.26	0.87	1.00	1.00	0.84	1.14	1.18	0.92	1.27	1.31	0.37	1.19	1.58"
4	0.09	0.42"	0.92"	1.09	1.11	1.05	1.09	1.22	1.14	1.14	1.40	1.14	0.54	1.55	1.30"
8	0.55	0.88	0.47	1.31	1.11	0.72	1.26	1.44	0.92	1.27	2.03	1.23	0.07	0.05	0.12

s.e.: T1 = 0.047; T2 = 0.056; T3 = 0.026

[1] see Table A3.1

Table A3.11: Herbage Cu concentration of PRG grown in soils 1 to 9 at three lime levels.

Soil	*Herbage Cu concentration (mg kg^{-1} DM)*								
	Unlimed			Lime level 2			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3
1	12.7	9.1	8.4"	9.9	7.3	7.6	9.7	7.3	6.0
2	10.7	8.8	11.0	9.1"	7.2	8.3	11.1"	8.4	8.0
3	6.7	6.7"	9.1	5.2	5.6	8.0	4.5	4.0	5.4
4	6.4	7.5"	8.0	6.6	5.0	6.6	6.1"	3.2	3.3"
5	6.7	6.7	8.1	7.1	6.7	8.3	8.2	6.0	5.2
6	8.8	5.8	8.3	7.4	7.7	7.6	6.1"	4.0	3.3
7	3.4	3.3	6.1	4.0	3.9	5.4	3.4	3.8	2.8
8	2.4	1.4	2.6	2.5"	2.2	3.4	2.9	0.5	1.7
9	5.4	6.0	6.7	5.3	6.1	7.1	5.8	4.7"	6.2

s.e.: T1 = 0.40; T2 = 0.28; T3 = 0.29

[1]see Table A3.1

Table A3.12: Herbage Cu concentration of PRG grown in soils 2, 4 and 8 at five lime levels.

Soil	*Herbage Cu concentration (mg kg^{-1} DM)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	10.7	8.9	11.0	9.7	7.7	9.0	10.0	7.7	8.3	11.5	8.7"	8.2"	11.2"	9.3	8.0
4	6.4	7.4"	8.0	7.5	7.3	7.6	7.4	6.2	6.2"	8.4	6.2	5.6	6.8	4.7	3.2"
8	2.4	2.2	2.6	2.3	3.4	3.9	3.0	3.0	3.4	2.6	3.0	2.8	2.9	-	1.7

s.e.: T1 = 0.56; T2 = 0.23; T3 = 0.23

[1]see Table A3.1

Table A3.13: Herbage Mo concentration of PRG grown in soils 1 to 9 at three lime levels.

Soil	*Herbage Mo concentration (mg kg^{-1} DM)*								
	Unlimed			Lime level 2			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3
1	0.94	1.0	1.4	0.96	1.3	2.2	1.9	3.1	4.1
2	1.3	0.34	1.2	2.2	8.2"	6.0"	4.0	4.8"	8.0"
3	1.0	2.3	8.5	0.88	2.0	5.6"	3.4	6.3"	5.7
4	0.44	0.82	1.5	0.66	1.9	3.6	1.4"	1.6	2.7
5	0.18	0.34	0.92	0.81	1.4	1.3	2.9	2.1	4.4
6	0.43	0.36	1.0	0.87	1.8	3.1	1.3	1.4	3.1
7	0.27	0.78	2.4	0.62	1.0	2.8	1.2	1.7	2.0
8	0.48"	0.90	1.2	0.84	3.5	5.0	0.71	0.23	4.1
9	0.75	0.90	2.8	0.70	1.0	2.7	2.8	2.3	3.9"

s.e.: T1 = 0.214; T2 = 0.460; T3 = 0.657

[1]see Table A3.1

Table A3.14: Herbage Mo concentration of PRG grown in soils 2, 4 and 8 at five lime levels.

Soil	*Herbage Mo concentration (mg kg^{-1} DM)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	1.3	0.35	1.2	1.3	2.0	3.1"	2.0	5.4	6.1"	6.0	6.9	10.2"	4.0	5.8	8.1"
4	0.44	0.82	1.5	0.68	1.8	3.3	0.79	2.0	3.6	1.3"	1.8	2.7	1.8	1.4	2.7
8	0.56"	0.40	1.2	0.76	2.6	5.4	1.2	2.9	5.0	1.5	2.4	4.3	0.71	0.23	4.1

s.e.: T1 = 0.287; T2 = 0.720; T3 = 0.660

[1] see Table A3.1

Table A3.15: Herbage S concentration of PRG grown in soils 1 to 9 at three lime levels.

Soil	*Herbage S concentration (% DM)*								
	Unlimed			Lime level 2			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3
1	0.32	0.30	0.28	0.36"	0.29	0.37	0.34	0.32	0.31
2	0.28	0.30	0.26	0.41	0.33	0.32	0.39	0.30	0.25
3	0.22	0.10	0.10	0.21	0.10	0.10	0.29	0.21	0.12
4	0.37	0.30	0.22	0.40	0.26	0.19	0.54	0.33	0.26
5	0.32	0.32	0.32	0.34	0.29	0.36	0.34	0.26	0.31
6	0.32	0.14	0.14	0.24	0.16	0.13	0.45"	0.24	0.25
7	0.30	0.11	0.10	0.38	0.16	0.13	0.40	0.28"	0.23
8	0.35	0.18	0.10	0.36	0.14	0.11	0.29	0.39	0.38
9	0.29	0.14	0.11	0.28	0.14	0.12	0.33	0.28	0.30

s.e.: T1 = 0.012; T2 = 0.010; T3 = 0.011

[1]see Table A3.1

Table A3.16: Herbage S concentration of PRG grown in soils 2, 4 and 8 at five lime levels.

Soil	*Herbage S concentration (% DM)*														
	Unlimed			Lime level 1			Lime level 2			Lime level 3			Lime level 4		
	[1]T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
2	0.28	0.30	0.26	0.29"	0.26	0.29	0.36	0.30	0.32	0.38	0.30	0.30	0.39	0.25"	0.25
4	0.37	0.30	0.22	0.32	0.22	0.15	0.34	0.24	0.19	0.42	0.27"	0.24	0.54	0.30	0.26
8	0.35	0.17	0.10	0.26	0.10	0.10	0.33	0.12	0.11	0.43	0.17"	0.12	0.29	0.39	0.38

s.e.: T1 = 0.013; T2 = 0.009; T3 = 0.010

[1]see Table A3.1

Appendix 3.2: Data from Experiment 2.

Table A3.17: pH of soils 2, 4 and 8 in which PRG or WC was grown at three lime levels and, for PRG, with two methods of lime application.

Soil	Sampling time [1]	pH								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	0	4.4	5.4	6.7	4.5	5.5	6.7	5.8	7.2··	7.8
	1	4.3	5.0	6.2	4.3	5.1	6.3	4.6	5.6	6.8
	2	4.2	5.0	6.3	4.3	5.0	6.3	4.2··	4.8	6.3
4	0	4.5	5.6··	6.4	4.6	5.5	6.6	6.6	7.3	7.8
	1	4.4	5.3	6.3	4.5	5.3··	6.1	4.8	6.0	6.5
	2	4.3··	5.0	6.0··	4.4	5.2	6.1	4.1	5.2··	6.0
8	0	4.3	5.2	6.3··	4.4	5.4	6.4	6.0	7.2	7.7
	1	4.2	5.0	6.0	4.2	5.1	6.0··	4.6	5.8	6.4
	2	4.1	4.8	5.8	4.2	5.0	5.9	4.0	4.8	6.3··

s.e. Part A (Lime A): T0 = 0.05; T1 = 0.04; T2 = 0.03

Part B (PRG): T0 = 0.04; T1 = 0.08; T2 = 0.05

[1]Sampling time 0, 1, 2: days 0, 40 and 70 - start of experiment, first and second harvests (T0, T1, T2).

[2]A1, A2, A3: lime applied throughout soil at levels 1, 2 and 3.

S1, S2, S3: lime applied to surface layer (top 30%) of soil at levels 1, 2, 3.

Table A3.18: DM production of PRG and WC grown in soils 2, 4 and 8 at three lime levels and, for PRG, with two methods of lime application.

Soil	Harvest time [1]	Herbage DM production (g pot^{-1})								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	0.76	0.85	0.16	1.66	2.01	2.41	1.52	1.88	0.26
	2	1.50	1.86	1.29··	1.38	1.53	1.84	1.32	1.52	1.59··
4	1	1.05	1.04	0.58	2.13	2.43	2.45	1.95	1.87	1.33
	2	1.66	1.77	2.06	1.41	1.55	1.66	1.37	1.38	1.50
5	1	1.04	1.29	0.80	2.38	2.67	2.93	2.42	2.24	0.65··
	2	1.87	2.35	2.51	1.26	1.49	1.89	1.27	1.53	1.64

s.e. Part A (Lime A): Harvest 1 = 0.073; Harvest 2 = 0.057

Part B (PRG): Harvest 1 = 0.071; Harvest 2 = 0.038

[1] Harvest 1, 2: days 40 and 70.

[2] A1, A2, A3: lime applied throughout soil at levels 1, 2 and 3.

S1, S2, S3: lime applied to surface layer (top 30%) of soil at levels 1, 2, 3.

Table A3.19: Herbage Cu concentration of PRG and WC grown in soils 2, 4 and 8 at three lime levels and, for PRG, with two methods of lime application.

Soil	Harvest time[1]	*Herbage Cu (mg kg^{-1} DM)*								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	8.7"	9.4	8.6	6.8	6.6	7.9	7.7	7.8	11.4
	2	7.6	8.3"	8.5	4.6	4.4	4.7	4.6	4.6	8.0"
4	1	7.8	6.8	7.9	5.6	5.0	5.9	5.5	4.8	5.6
	2	12.4"	13.0"	6.3	5.0	3.2	3.6	5.0	4.7	3.8
8	1	2.0	2.2	2.7	2.5	2.4	2.4	1.8	1.7	1.8
	2	2.0"	2.1	1.3	2.2	1.9	1.7	1.4	1.2	1.2

s.e. Part A (Lime A): Harvest 1 = 0.36; Harvest 2 = 0.16
Part B (PRG): Harvest 1 = 0.41; Harvest 2 = 0.19

[1]Harvest 1, 2: days 40 and 70.

[2]A1, A2, A3: lime applied throughout soil at levels 1, 2 and 3.

S1, S2, S3: lime applied to surface layer (top 30%) of soil at levels 1, 2, 3.

Table A3.20: Herbage Mo concentration of PRG and WC grown in soils 2, 4 and 8 at three lime levels and, for PRG, with two methods of lime application.

Soil	Harvest time[1]	*Herbage Mo (mg kg^{-1} DM)*								
		WC			PRG					
		[2]A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	2.1	3.3	13.8	1.9	4.0	7.4	4.6	4.7"	4.2
	2	2.6	2.7	11.9"	2.3	5.0	8.9	4.6	8.5	5.0
4	1	1.8	2.4	3.7"	1.9	1.8	2.1	1.6	2.1	1.6
	2	3.5	3.2	2.5	3.0	2.8	2.4	1.7	1.8	1.4
8	1	1.1	2.1	5.1	1.7	1.9	2.6	1.5	1.8	1.5
	2	1.3	1.3"	1.4	3.1	3.8	2.3	2.1	1.4	1.0

s.e. Part A (Lime A): Harvest 1 = 0.57; Harvest 2 = 0.48
Part B (PRG) Harvest 1 = 0.52; Harvest 2 = 0.43

[1,2] as for Table A3.19

Table A3.21: Herbage S concentration of PRG and WC grown in soils 2, 4 and 8 at three lime levels and, for PRG, with two methods of lime application.

Soil	Harvest time[1]	*Herbage S concentration (% DM)*								
		WC			PRG					
		[2] A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	0.20	0.21	0.22	0.23	0.25	0.31	0.27	0.28	0.36
	2	0.14	0.15	0.17"	0.22	0.26	0.26	0.20	0.18	0.23"
4	1	0.21	0.21	0.23	0.22	0.24	0.28"	0.24	0.27	0.30"
	2	0.16	0.16	0.13	0.15	0.16	0.20	0.14	0.18	0.22
8	1	0.17	0.20	0.23	0.14	0.17	0.23	0.14	0.20	0.33"
	2	0.09	0.10	0.12	0.08	0.08	0.09	0.06	0.08	0.16

s.e. Part A (Lime A): Harvest 1 = 0.008; Harvest 2 = 0.005

Part B (PRG): Harvest 1 = 0.011; Harvest 2 = 0.008

[1,2] as for Table A3.19

Table A3.22: Herbage Ca concentration of PRG and WC grown in soils 2, 4 and 8 at three lime levels and, for PRG, with two methods of lime application.

Soil	Harvest time[1]	*Herbage Ca concentration (% DM)*								
		WC			PRG					
		[2] A1	A2	A3	A1	A2	A3	S1	S2	S3
2	1	1.7	1.8	1.9	0.56	0.62	0.66	0.53	0.56	0.46
	2	1.6	1.9	1.4	0.55	0.58	0.57	0.46	0.48	0.37
4	1	1.8	1.8	1.6"	0.62	0.58	0.59	0.49	0.44	0.33
	2	2.1"	2.0	1.6	0.68	0.68	0.67	0.56	0.48	0.34
8	1	1.2	1.6	1.4	0.54	0.53	0.51	0.37	0.38	0.30
	2	1.2	1.7	1.4	0.78	0.79	0.73	0.56	0.55	0.33

s.e. Part A (Lime A): Harvest 1 - WC = 0.05; PRG = 0.015
Harvest 2 - WC = 0.04; PRG = 0.016

Part B (PRG): Harvest 1 = 0.012; Harvest 2 = 0.015

[1,2] as for Table A3.19

Appendix 3.3: Data from Experiment 3.

Table A3.23: pH for soils 2 and 8 before and after 42 days growth of PRG with N applied as urea.

Soil	*pH*			
	40 kg N ha^{-1}		80 kg N ha^{-1}	
	†T0	T1	T0	T1
2	5.5	4.9	5.7	4.9
8	5.5	4.9	5.4	4.9

s.e.: T2 = 0.05; T1 = 0.02

†T_0 , T_1 : sampling days 0 and 42, i.e. start and conclusion of experiment.

Table A3.24: Herbage DM production for PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N, NO_3^--N and urea.

Soil	*Herbage dry matter production (g pot^{-1})*						
	No N	NO_3^--N		NH_4^+-N		Urea	
		†40	80	40	80	40	80
2	0.58	1.60	2.51··	1.48	2.29	1.48	2.24
8	2.27	2.80	3.02	2.68	2.90	2.70	2.83

s.e.: No nitrogen = 0.028; plus nitrogen = 0.040

†40, 80 = kg N ha^{-1} applied.

Table A3.25: Herbage Cu concentration for PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N, NO_3^--N and urea.

Soil	*Herbage Cu concentration (mg kg^{-1} DM)*						
	No N	NO_3^--N		NH_4^+-N		Urea	
		†40	80	40	80	40	80
2	5.5··	4.8··	5.5	5.4	5.4	5.2	5.4
8	1.8	1.8	1.8	1.8	1.6	1.8	1.6

s.e.: No nitrogen = 0.08; plus nitrogen = 0.11

†see Table A3.25

Table A3.26: Herbage Mo concentration for PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N, NO_3^--N and urea.

Soil	*Herbage Mo concentration (mg kg^{-1} DM)*						
	No N	NO_3^--N		NH_4^+-N		Urea	
		†40	80	40	80	40	80
2	4.3··	2.1	2.9	2.9	2.4	2.1	2.4
8	2.2	2.4	2.2	1.9	2.2	1.8	2.6

s.e.: No nitrogen = 0.32; plus nitrogen = 0.45

†see Table A3.25

Table A3.27: Herbage S concentration for PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N, NO_3^--N and urea.

Soil	*Herbage S concentration (% DM)*						
	No N	NO_3^--N		NH_4^+-N		Urea	
		†40	80	40	80	40	80
2	0.38	0.28	0.23	0.33	0.32	0.33	0.32
8	0.15	0.13	0.12	0.14	0.14	0.14	0.14

s.e.: No nitrogen = 0.005; plus nitrogen = 0.007

†see Table A3.25

Table A3.28: Herbage N concentration for PRG grown for 42 days in soils 2 and 8 with and without additional N, applied as NH_4^+-N, NO_3^--N and urea.

Soil	*Herbage N concentration (% DM)*						
	No N	NO_3^--N		NH_4^+-N		Urea	
		†40	80	40	80	40	80
2	1.2	1.1	1.4	1.2	1.5	1.2	1.4
8	1.3	1.5	1.9	1.8	2.0	1.6	2.1

s.e.: No nitrogen = 0.03; plus nitrogen = 0.04

†see Table A3.25

Appendix 3.4: Data from Experiment 4.

Table A3.29: Extractable Cu concentrations of soils 2 and 8 in which PRG or WC was grown for 52 days at two lime levels and three P-levels.

Soil	Lime level	*Extractable soil Cu (mg kg^{-1} air-dry soil)*								
		†T0			T2 - WC			T2 - PRG		
		²P1	P2	P3	P1	P2	P3	P1	P2	P3
2	1	3.4	3.2	3.2	4.5	4.4	4.4	4.7	4.6	4.6
	2	3.9	3.7	3.4	4.7	4.9	4.9	5.0	5.0	5.0
8	1	0.40	0.42	0.42	0.54	0.51	0.50	0.58	0.59	0.51
	2	0.50	0.48	0.45	0.56	0.56	0.55	0.58	0.55"	0.54

s.e.: T0 = 0.12; T2 - soil 2 = 0.07, soil 8 = 0.014

†T0, T2-WC, T2-PRG = day 0 and day 52 after WC or PRG growth, i.e. start and conclusion of experiment.

²P1, P2, P3 = phosphorus levels 1, 2 and 3 equivalent to 24, 48 and 96 kg P ha^{-1}

Table A3.30: Extractable Mo concentrations of soils 2 and 8 in which PRG or WC was grown for 52 days at two lime levels and three P-levels.

Soil	Lime level	*Extractable soil Mo concentration (mg kg^{-1} air-dry soil)*								
		†T0			T2 - WC			T2 - PRG		
		²P1	P2	P3	P1	P2	P3	P1	P2	P3
2	1	0.043	0.043	0.085	0.056	0.057	0.051	0.045	0.047	0.050
	2	0.087	0.130	0.133	0.082	0.075	0.077	0.065	0.073	0.070
8	1	0.043	0.047	0.042	0.034	0.036	0.035	0.027	0.020	0.031
	2	0.047	0.032	0.018	0.032"	0.039	0.027	0.019	0.024	0.023

s.e.: T0 = 0.0118; T2 = 0.0049

†,² see Table A3.29

Table A3.31: Extractable SO_4^{2-}-S concentrations of soils 2 and 8 in which PRG or WC was grown for 52 days at two lime levels and three P-levels.

Soil	Lime level	*Extractable soil SO_4^{2-}-S concentration (mg kg^{-1} air-dry soil)*								
		†T0			T2 - WC			T2 - PRG		
		²P1	P2	P3	P1	P2	P3	P1	P2	P3
2	1	49	52	53	48	40	32	18	19	17
	2	60	67	61	82	74	65	24	21	26
8	1	41	40	39	32	19	13	8	11	8
	2	53	50	58	78	66	43"	10	12	13

s.e.: T0 = 7.0; T2 = 2.6

† ² see Table A3.29

Table A3.32: Extractable Ca concentrations of soils 2 and 8 in which PRG or WC was grown for 52 days at two lime levels and three P-levels.

Soil	Lime level	*Extractable soil Ca concentration (mg kg^{-1} air-dry soil)*								
		†T0			T2 - WC			T2 - PRG		
		²P1	P2	P3	P1	P2	P3	P1	P2	P3
2	1	820	840	915	850	850	870	840	825	890
	2	2420	2560	2380	2490	2480	2490	2490	2470	2490
8	1	1610	1500	1650	1590	1570	1640	1550	1530	1630
	2	3900	3860	4000	3860	3750	3940	3770	3820	3870

s.e.: T0 = 68; T2 = 37

†,² see Table A3.29

Table A3.33: Extractable PO_4^{3-}-P concentrations of soils 2 and 8 in which PRG or WC was grown for 52 days at two lime levels and three P-levels.

Soil	Lime level	*Extractable soil PO_4^{3-}-P conc. (mg kg^{-1} air-dry soil)*								
		†T0			T2 - WC			T2 - PRG		
		²P1	P2	P3	P1	P2	P3	P1	P2	P3
2	1	2.6	3.8	7.6	2.6	3.9	7.0	2.7	3.1	5.9
	2	2.4	4.2	7.8	2.0	2.9	6.6	1.5	2.3	4.6
8	1	4.5	6.6	11.7	4.3	5.8	9.6	2.6	3.4	7.5
	2	5.6	8.1	14.2	5.0	7.1	10.8	3.8	5.3	8.4

s.e.: T0 = 1.29; T2 = 0.34

†,²see Table A3.29

Table A3.34: Herbage DM production for PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage DM production (g pot^{-1})*					
			WC			PRG		
			[2]P1	P2	P3	P1	P2	P3
2	1	1	0.23	0.42	0.97	1.71	2.13**	2.39
		2	0.46	0.91**	1.15	1.09	1.19	1.20
	2	1	0.16	0.32	0.52	1.86	2.51**	2.69**
		2	0.37	0.72	1.34**	1.54	1.55	1.50
8	1	1	0.35	0.60	1.27	2.29	2.38	2.39
		2	0.66	0.91	1.25	1.12	1.17	1.14
	2	1	0.13	0.14	0.61	2.32	2.51	2.85
		2	0.37	0.54	1.38**	1.71	1.70	1.72

s.e.: Harvest 1 = 0.102; Harvest 2 = 0.060

†Harvest 1, 2 = days 36 and 52

[2]P1, P2, P3 = phosphorus levels 1, 2 and 3 equivalent to 24, 48 and 96 kg P ha^{-1}.

Table A3.35: Herbage Cu concentration of PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage Cu concentration (mg kg^{-1} DM)*					
			WC			PRG		
			[2]P1	P2	P3	P1	P2	P3
2	1	1	7.3	7.8	7.6	6.7	6.6	5.0
		2	10.9	8.7	9.4	7.5	7.0	7.2
	2	1	7.7	8.8	9.5**	9.7	6.8**	6.0
		2	8.3	8.0	8.5	7.1	6.5**	6.2**
8	1	1	4.4	2.5	2.0	2.0	2.0	1.8
		2	2.2	2.1	2.0	2.7	2.7	2.6
	2	1	2.8	3.0	2.3	2.8	2.5	2.1
		2	2.3	2.2	1.7	2.1**	2.4	2.4

s.e.: Harvest 1 = 0.42; Harvest 2 - soil 2 = 0.24, soil 8 = 0.11

† [2] see Table A3.34

Table A3.36: Herbage Mo concentration of PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage Mo concentration (mg kg^{-1} DM)*					
			WC			PRG		
			²P1	P2	P3	P1	P2	P3
2	1	1	1.6	3.6	2.0	2.1	1.4	1.2
		2	3.0	4.1	4.4	3.6"	3.5	4.7
	2	1	22.0	15.0	41.2"	5.3	4.0	3.0
		2	19.2	17.4	16.3"	8.4	6.6	5.7
8	1	1	2.7	3.6	3.0	2.2	1.9	1.5
		2	2.8	2.7	1.9	3.5	4.6	4.8
	2	1	11.3	14.9	11.8	2.6"	2.0	2.0
		2	9.0	5.0	3.1	3.6	2.6	2.5

s.e.: Harvest 1 = 1.11; Harvest 2 = 1.04

†,² see Table A3.34

Table A3.37: Herbage S concentration of PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage S concentration (% DM)*					
			WC			PRG		
			²P1	P2	P3	P1	P2	P3
2	1	1	0.23	0.23	0.20	0.40	0.34"	0.31
		2	0.27	0.19	0.18	0.34	0.28	0.28
	2	1	0.23	0.28	0.30	0.56	0.43"	0.40
		2	0.22	0.23	0.21	0.34"	0.31	0.29
8	1	1	0.28	0.28	0.20	0.23	0.21"	0.22
		2	0.22	0.21	0.14	0.10"	0.11	0.12
	2	1	0.28	0.30	0.28	0.39	0.34	0.28
		2	0.25	0.29	0.26	0.13	0.14	0.13

s.e.: Harvest 1 = 0.018; Harvest 2 = 0.014

†,²see Table A3.34

Table A3.38: Herbage Ca concentration of PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage Ca concentration (% DM)*					
			WC			PRG		
			²P1	P2	P3	P1	P2	P3
2	1	1	1.12	1.29	1.53"	0.36	0.36	0.36
		2	1.09	0.98	1.07	0.42	0.42	0.42
	2	1	1.26	1.30	1.47	0.43	0.46	0.43
		2	1.20	1.30	1.28"	0.38"	0.42	0.40
8	1	1	0.89	0.89	0.90	0.35	0.40	0.39
		2	0.84	0.84	0.80	0.47	0.51	0.57
	2	1	0.85	0.90	0.97	0.39	0.36	0.44"
		2	0.85	0.96	0.95	0.45	0.51	0.50

s.e.: Harvest 1 - WC = 0.036, PRG = 0.037
Harvest 2 - WC = 0.040, PRG = 0.025

†,²see Table A3.34

Table A3.39: Herbage P concentration of PRG and WC grown in soils 2 and 8 at two lime levels and three P-levels.

Soil	Lime level	Harvest†	*Herbage P concentration (% DM)*					
			WC			PRG		
			²P1	P2	P3	P1	P2	P3
2	1	1	0.10	0.11	0.13	0.11	0.17	0.20
		2	0.19	0.16	0.21	0.14	0.20	0.30
	2	1	0.10	0.14	0.17	0.14	0.15"	0.19
		2	0.13	0.13	0.16	0.14"	0.16"	0.23
8	1	1	0.12	0.14	0.14	0.18	0.27	0.34"
		2	0.12	0.17"	0.24	0.30"	0.39	0.48
	2	1	0.13	0.18	0.20	0.18	0.22	0.28"
		2	0.16	0.20	0.20	0.26	0.24	0.34

s.e.: Harvest 1 = 0.007; Harvest 2 = 0.010

†,² see Table A3.34

Appendix 3.5: Data from Experiment 5.

Table A3.40: Herbage dry matter production of PRG and WC grown in soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

Soil	Harvest†	*Herbage dry matter production (g pot^{-1})*			
		WC		PRG	
		No Cu	+ Cu	No Cu	+ Cu
2	1	0.34	0.33	0.98	1.03
	2	0.45	0.42	1.27	1.26
8	1	0.59	0.73	1.36	1.42
	2	0.54	0.70	1.52	1.56

s.e.: Harvest 1 = 0.032; Harvest 2 = 0.036

†Harvest 1, 2: days 30 (PRG) / 38 (WC) and 50

Table A3.41: Herbage Cu concentration of PRG and WC grown in soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

Soil	Harvest†	*Herbage Cu concentration (mg kg^{-1} DM)*			
		WC		PRG	
		No Cu	+ Cu	No Cu	+ Cu
2	1	11.7	12.1	11.1	15.3"
	2	13.9	14.7	7.5	11.2
8	1	2.7	13.2"	2.8	16.5"
	2	3.5	15.4"	2.5	8.8"

s.e.: Harvest 1 = 0.25; Harvest 2 = 0.42

†see Table A3.40.

Table A3.42: Herbage Mo concentration of PRG and WC grown in soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

Soil	Harvest†	*Herbage Mo concentration (mg kg^{-1} DM)*			
		WC		PRG	
		No Cu	+ Cu	No Cu	+ Cu
2	1	4.1	7.6	7.2	7.1
	2	3.2	5.0	15.5	13.1
8	1	5.2	10.0"	3.2	4.0
	2	3.3	23.3×	6.9	6.6

s.e.: Harvest 1 = 1.48; Harvest 2 = 2.59 (excluding value ×)

†see Table A3.40.

Table A3.43: Herbage S concentration of PRG and WC grown in soils 2 and 8 with and without added Cu (10 kg Cu ha^{-1}).

Soil	Harvest†	*Herbage S concentration (mg kg^{-1} DM)*			
		WC		PRG	
		No Cu	+ Cu	No Cu	+ Cu
2	1	0.28	0.28	0.44"	0.44
	2	0.27	0.27	0.26	0.28
8	1	0.30	0.28	0.36	0.34
	2	0.25	0.24	0.12	0.11

s.e.: Harvest 1 = 0.012; Harvest 2 = 0.006

†see Table A3.40.

Appendix 6: Data from Experiment 6.

Table A3.44: pH of 'fresh' soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	pH (fresh soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	3.6	3.8	3.7	3.7	3.7
		1	5.9	6.0	5.8	5.5	5.5
	2	0	3.5	3.9	4.6	5.3"	5.0
		1	6.0	6.0	6.1	6.3	6.2
8	1	0	3.3	3.6	3.5	3.5	3.6
		1	6.0	6.1	6.0	5.9	5.8
	2	0	3.4	3.6	3.7	3.8	3.8
		1	5.8	6.0	5.8	5.8	5.8
		s.e.:	0.02	0.03	0.04	0.05	0.03

†1,2: 60% FC, waterlogged.

²0,1: unlimed, limed to pH 6.0.

Table A3.45: Extractable Cu concentrations of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	Extractable soil Cu (mg kg^{-1} oven-dry soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	2.3	2.3	2.3	2.2	2.2
		1	2.1	2.3	2.1	1.9	1.9
	2	0	2.0	1.9	1.1	1.3	0.9
		1	1.9	1.3	1.0	1.4	1.2"
8	1	0	0.32	0.27	0.25	0.23	0.30
		1	0.30	0.28	0.27	0.25	0.28
	2	0	0.29	0.25	0.24	0.28	0.16
		1	0.30	0.21	0.27	0.28	0.30
	s.e.	Soil 2 :	0.03	0.07	0.04	0.05	0.05
		Soil 8 :	0.008	0.012	0.014	0.014	0.011

†,² see Table A3.44

Table A3.46: Extractable Mo concentrations of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	Extractable soil Mo (mg kg^{-1} oven-dry soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	0.028	0.028	0.022	0.019	0.030
		1	0.041	0.060	0.051	0.065	0.096
	2	0	0.023	0.034	0.041	0.061	0.135
		1	0.049	0.066	0.059	0.125	0.180
8	1	0	0.040	0.036	0.026	0.029	0.033
		1	0.050	0.038··	0.035	0.041	0.039
	2	0	0.033	0.045	0.036	0.036	0.042
		1	0.044	0.036	0.030	0.031	0.035
	s.e. Soil 2:		0.0034	0.0024	0.0037	0.0073	0.0227
	Soil 8:		0.0054	0.0011	0.0020	0.0054	0.0056

†, ² see Table A3.44

Table A3.47: Extractable SO_4^{2-}-S concentrations of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	Extractable soil SO_4^{2-}-S (mg kg^{-1} oven-dry soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	25	26	29	24	28
		1	48	62	62	52	60
	2	0	18	23	14	2.6	<1.5
		1	26	48	<1.5	<1.5	<1.5
8	1	0	19··	27	26	24	28··
		1	37	49	66	61	74
	2	0	14	20	23··	10··	18
		1	34	32	10	9	10
		s.e.:	1.4	1.7	2.0	1.4	1.1

†, ² see Table A3.44

Table A3.48: Extractable NH_4^+-N concentrations of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	Extractable soil NH_4^+-N (mg kg^{-1} oven-dry soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	25	25	20	10	12
		1	43	66	91	<1.0	<1.0
	2	0	17	41	46	30	53
		1	33	60	79	56	100
8	1	0	100	140	150	165	230
		1	120	290"	520	350"	370
	2	0	45	120	125"	150	200
		1	88	160	165"	200	265
	s.e. Soil 2:		2.2	5.8	3.1	1.7	6.5
	Soil 8:		2.0	8.4	13.8	10.5	5.7

†, ² see Table A3.44

Table A3.49: Extractable NO_3^--N concentrations of soils 2 and 8 incubated for three months at 20°C either unlimed or with added lime and maintained at 60% FC or waterlogged.

Soil	Moisture† regime	Lime² level	Extractable soil NO_3^--N (mg kg^{-1} oven-dry soil) Day 3	Day 10	Day 24	Day 53	Day 94
2	1	0	<1	<1	1.5	2.4	7.5
		1	6	10	20	105	100
	2	0	<1	<1	2.1	<1	<1
		1	13	15	13	10	16
8	1	0	<1	<1	<1	<1	<1
		1	25	46	18	17	99
	2	0	<1	<1	<1	<1	<1
		1	25	38	20	11	22
	s.e. Lime 0:		-	-	-	0.64	0.79
	Lime 1:		2.0	5.8	2.8	3.0	9.6

†, ² see Table A3.44

Table A3.50: Herbage Cu concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	Herbage Cu concentration (mg kg^{-1} DM) †Dry	Moist	Waterlogged
2	60	9.8	8.9	11.9
	100	6.3	6.9	11.0"
8	60	2.2	1.8	2.9
	100	1.8	1.6	2.6

s.e.: Soil 2 = 0.29
Soil 8 = 0.21

†Soil pre-treatment

Table A3.51: Herbage Mo concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	Herbage Mo concentration (mg kg^{-1} DM) †Dry	Moist	Waterlogged
2	60	3.7	2.7	2.2
	100	3.8	2.8	2.0
8	60	1.4"	0.94	1.3"
	100	1.2"	0.67	0.82

s.e.: Soil 2 = 0.27
Soil 8 = 0.067

†Soil pre-treatment

Table A3.52: Herbage S concentration of PRG grown in soils 2 and 8 for 36 days at 60% or 100% FC after the soils had been stored dry, moist or waterlogged for three months.

Soil	Watering regime (% FC)	Herbage S concentration (% DM) †Dry	Moist	Waterlogged
2	60	0.31	0.27	0.30
	100	0.26	0.21	0.27"
8	60	0.26	0.28	0.20"
	100	0.18	0.25	0.11

s.e.: 0.006

†Soil pre-treatment